Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Aqueous extract from the germinated seeds of sweet *Lupinus albus*

EC Number: -
CAS Number: -

CLH-O-0000007261-81-01/F

Adopted

16 March 2023

16 March 2023

CLH-O-0000007261-81-01/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of Regulation (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemical name: **Aqueous extract from the germinated seeds of sweet *Lupinus albus***

EC Number: **-**

CAS Number: **-**

The proposal was submitted by **The Netherlands** and received by RAC on **17 February 2022**.

In this opinion, all classification and labelling elements are given in accordance with the CLP Regulation.

PROCESS FOR ADOPTION OF THE OPINION

The Netherlands has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation/*** on **21 March 2022**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **20 May 2022**.

ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Normunds Kadikis**

Co-Rapporteur, appointed by RAC: **Anja Menard Srpčič**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was adopted on **16 March 2023** by **consensus**.

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	Chemical name	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors and ATE	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	No current Annex VI entry										
Dossier submitters proposal	TBD	Aqueous extract from the germinated seeds of sweet *Lupinus albus*									
RAC opinion	TBD	Aqueous extract from the germinated seeds of sweet *Lupinus albus*									
Resulting Annex VI entry if agreed by COM	TBD	Aqueous extract from the germinated seeds of sweet *Lupinus albus*									

GROUNDS FOR ADOPTION OF THE OPINION

RAC general comment

Aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20 % of BLAD as the lead component. BLAD is a naturally occurring polypeptide formed during day four to twelve of the germination process of sweet *Lupinus albus*. BLAD binds to chitin and chitosan of fungi which weakens the cell wall structure. It has been found to be a very effective fungicide against powdery mildew and other diseases.

Filtered and concentrated liquid of aqueous extract from the germinated seeds of sweet *Lupinus albus* is known with a trademark Problad Plus. The test article in question was administered as received with minor exceptions.

All studies referred to were performed according to OECD Test Guidelines (TG) and GLP.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

The Dossier Submitter (DS) considered the aqueous extract from the germinated seeds of sweet *Lupinus albus* as not explosive based on an obsolete test method involving an investigation on the effects of heat (flame) and shock (impact hammer) as well as on theoretical considerations outlined in Appendix 6 (screening procedures) of the UN Recommendations on the transport of dangerous goods, manual for tests and criteria, rev. 6.

The DS considered that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not a flammable liquid. According to the test method EEC A.9 / OPPTS 830.700 (closed cup), the flashpoint of the aqueous extract from the germinated seeds of sweet *Lupinus albus* is > 100 °C (Wo, 2012b) not meeting the CLP criterion for flammability.

The DS considered the aqueous extract from the germinated seeds of sweet *Lupinus albus* as not self-reactive nor pyrophoric liquid. The justification for these conclusions is based on the facts that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is an aqueous solution, it is stable when heated to its boiling point and it is not flammable.

The DS considered the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not expected to be self-heating due to its stability when heated to its boiling point. In addition, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not flammable.

The DS considered the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not oxidising according to the study performed by Wo (2012b). No oxidising or reducing potential has been reported except when tested with potassium permanganate where a reaction was observed within two minutes. In addition, it was considered that the extract does not contain groups that would imply oxidising properties such as nitrates, metal oxides, hypofluorites, difluoroaminopolynitroaryls, perchlorates, bromates and iodites.

With respect to corrosivity to metals, the DS considered that there are insufficient data to compare them to the CLP criteria. The pH of the aqueous extract from the germinated seeds of sweet *Lupinus albus* is approximately 6 and it is not classified with regard to skin and eye irritation. It was therefore not expected to be corrosive, but as metal corrosion is a complex process which is difficult to predict, a firm conclusion on classification was not made and the DS considered the classification inconclusive.

Comments received during consultation

No comments received during consultation.

Assessment and comparison with the classification criteria

RAC agrees that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not explosive. RAC supports the DS justification based on the fact that the aqueous extract from the germinated seeds of sweet *Lupinus albus* does not contain functional groups related to explosive properties and therefore it does not need to be further tested (Annex I to the CLP Regulation, 2.1.4.3). Even though the full composition of the extract is not known, the water content is high and it is assumed that all chemical groups associated with explosives properties but unsaturated hydrocarbons are not present in the aqueous extract from the germinated seeds of sweet *Lupinus albus*. If any unsaturated alkyl groups are present, they are expected to be present in fatty acids, which are known not to be explosive. Therefore, explosive properties are not expected and **no classification for explosive properties is warranted**.

RAC agrees that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not a flammable liquid as the CLP criterion for flammability (threshold of 60 °C or lower) is not met (Annex I to the CLP Regulation, table 2.6.1). **No classification as flammable liquid is warranted.**

RAC agrees with the DS that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not a self-reactive substance. RAC agrees that the CLP criteria for testing and the screening procedures set out in Annex I to the CLP Regulation (point 2.8.4.2) are not fully addressed. However, it is reasonable to assume that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not a self-reactive substance as the aqueous extract from the germinated seeds of sweet *Lupinus albus* is present in aqueous solution. Therefore, **no classification as a self-reactive substance is warranted**.

RAC agrees as well that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not pyrophoric liquid due to the experience in handling, in line with the CLP criteria (Annex I to the CLP Regulation, 2.9.4.1). Therefore, **no classification as a pyrophoric liquid is warranted**.

RAC agrees that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not expected to be self-heating based on its physical form (liquid). According to the CLP guidance (2.11.4.2), the property in question is generally pertinent to solid substances only. Therefore, **no classification as a self-heating substance is warranted**.

RAC is of opinion that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is not oxidising according to the study results provided by the DS (Wo, 2012b and Cage, 2013). RAC takes note of the considerations given by the DS that according to the CLP criteria (Annex I to the CLP Regulation, 2.13.4.1), if a substance or mixture contains compounds that contain oxygen, fluorine or chlorine bound only to carbon or hydrogen, oxidising properties do not need to be expected. However, the full composition of aqueous extract from the germinated seeds of sweet *Lupinus albus* was not fully identified, but it is of plant origin, and it is not expected that any unidentified compounds are present in the aqueous extract from the germinated seeds of sweet *Lupinus albus* that may induce oxidising properties. Therefore, **no classification for oxidizing properties is warranted**.

RAC agrees with the DS that **no classification for metal corrosion is warranted due to inconclusive data**.

HUMAN HEALTH HAZARD EVALUATION

RAC evaluation of acute toxicity

Summary of the Dossier Submitter's proposal

Acute oral toxicity

The DS provided one study on acute oral toxicity in rats conducted according to OECD TG 425 (CA 5.2.1/01, B.6.2.1, study 1, 2012). Three Sprague-Dawley female rats were administered with a limit dose of 5000 mg/kg bw by gavage. No clinical signs, effect on body weight or pathological findings were observed. Therefore, the oral LD_{50} is considered to be higher than 5000 mg/kg bw and no classification was proposed.

Acute dermal toxicity

The DS provided one study on acute dermal toxicity in rats conducted according to OECD TG 402 (CA 5.2.2/01, B.6.2.2, study 1, 2012). Five Sprague-Dawley female and 5 male rats were administered with a dose of 2000 mg/kg bw. No clinical signs, effect on body weight or pathological findings were observed. Therefore, the dermal LD_{50} is considered to be higher than 2000 mg/kg bw and no classification was proposed.

Acute inhalation toxicity

The DS provided one study on acute inhalation toxicity in rats conducted according to OECD TG 403 method (CA 5.2.3/01, B.6.2.3, study 1, 2012). Five Sprague-Dawley female and 5 male rats were administered with a dose of 5.34 mg/L (only nose). The test material was diluted in distilled water to a 50 % (w/w) mixture and used for generation of the test atmosphere using a ¼ inch atomiser. No clinical signs, effect on body weight or pathological findings were observed. Therefore, the inhalation LD_{50} is considered to be higher than 5.34 mg/L and no classification was proposed.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

Acute oral toxicity

According to CLP Regulation, a substance should be classified for acute oral toxicity if the oral LD_{50} is below 2000 mg/kg bw. In the available study the LD_{50} was higher than 5000 mg/kg bw and thus no classification is justified. RAC agrees with the DS's proposal for **no classification for acute oral toxicity**.

Acute dermal toxicity

According to CLP Regulation, a substance should be classified for acute dermal toxicity if the dermal LD_{50} is below 2000 mg/kg bw. In the available study the LD_{50} was higher than 2000 mg/kg bw and thus no classification is justified. RAC agrees with the DS's proposal for **no classification for acute dermal toxicity**.

Acute inhalation toxicity

According to CLP Regulation, a substance should be classified for acute inhalation toxicity if the inhalation LD_{50} is below 5 mg/L (dusts and mists). In the available study the LD_{50} was higher

than 5.34 mg/L and thus no classification is warranted. RAC agrees with the DS's proposal for **no classification for acute inhalation toxicity**.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The DS indicated that the only studies available to assess STOT SE are the acute toxicity studies via the oral, dermal and inhalation route described above.

No clinical signs, adverse pharmacologic effects or abnormal behaviour were observed during the acute oral toxicity study (see above). In addition, no effects on body weight or body weight gain were observed during the 14-day observation period following the single dosing at 5000 mg/kg bw. No other treatment-related pathological findings were observed.

With respect to the acute dermal toxicity study (see above), clinical signs of toxicity were limited to red nasal discharge on day 2 in 3/5 males and 1/5 females. No signs of dermal irritation were observed. In addition, no effects on body weight or body weight gain were observed during the 14-day observation period following the single dosing at 2000 mg/kg bw. No other treatment-related pathological findings were observed.

Concerning the acute inhalation toxicity study (see above), clinical signs were limited to one animal of each sex displaying signs of hypoactivity and 7 animals (3 males and 4 females) exhibiting abnormal respiration. All affected animals recovered by day 8 and appeared normal for the remainder of the observation period. No effects on body weight or body weight gain were observed during the 14-day observation period and no pathological findings were seen as well

No classification was proposed by the DS for STOT SE.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

RAC remarks that the only effects observed (signs of hypoactivity, abnormal respiration) were some clinical signs in the acute inhalation study following dosing at 5.34 mg/L. This value is higher than the guidance values given in the CLP Regulation for classification in Cat. 1 ($C \leq 1$ mg/L) or Cat. 2 ($5.0 \geq C > 1.0$ mg/L). Therefore, classification in Cat. 1 or Cat. 2 is not required.

The STOT SE Category 3 classification covers respiratory tract irritation and narcotic effects according to the criteria laid out in the CLP Regulation (Annex I, 3.8.2.2.1). In particular, it is mentioned that there are currently no validated animal tests that deal specifically with respiratory tract irritation, useful information may be obtained from the single and repeated inhalation toxicity tests in animals as a part of weight of evidence evaluation (Annex I to the CLP Regulation, 3.8.2.2.1.2(d)). However, when there are no data in human and animal data suggesting respiratory tract irritation effects, expert judgement is needed to estimate the severity of the effects observed in animals, the conditions of the test, the physical-chemical properties of the substance and whether those considerations alone might be sufficient for a classification in Category 3 for respiratory tract irritation.

For this substance, RAC notes that no human data are available that would indicate respiratory tract irritation.

In the acute inhalation toxicity study in rat, abnormal respiration (irregular respiration) was observed in 3/5 males and 4/5 females. In males, this clinical sign was observed on day 1-2 in 1 animal, and in the other two after removal of the exposure tube until day 2 to 4. In females, the irregular respiration was observed following removal of the exposure tube until day 1 for two animals, until day 3 for one animal and until day 7 for the 4th animal. No histopathological findings were observed in this study.

RAC considers justified the qualitative analysis performed by the DS that the aqueous extract from the germinated seeds of sweet *Lupinus albus* is a protein-based aqueous solution and, being a large molecule (> 200 kDa), volatilisation is not foreseen and its vapour pressure is expected to be low. Furthermore, RAC noted the hypothesis that the clinical signs observed in the acute inhalation study could be related to the stress suffered by the animals during and following the 4h nose-only exposure; however this was not supported by findings on the control group with pure air. In addition, the effect is not considered severe enough for classification for respiratory tract irritation.

RAC agrees with the DS's proposal that **no classification is warranted for STOT SE**.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The DS provided one study on skin corrosion/irritation performed with New Zealand White rabbits according to OECD TG 404 (CA 5.2.4/01, B.6.2.4, study 1, 2012). The aqueous extract from the germinated seeds of sweet *Lupinus albus* (0.5 mL) was administered to 3 male rabbits in semi-occlusive manner (see table below). Slight erythema was observed at 0.5 - 1 hour and 24 hours after patch removal, whereas no erythema was observed after 48 or 72 hours. In addition, slight oedema was observed at 0.5 - 1 hour after patch removal, but no oedema was detected in any of the animals after 24, 48 or 72 hours.

***Table**: Individual and mean skin irritation scores according to the Draize scheme*

Animal number	**Erythema**				**Oedema**				**Severity of irritation[1]**
	1	**2**	**3**	**Mean**	**1**	**2**	**3**	**Mean**	
after 0.5 – 1 hour	1	2	2	1.7	0	1	1	0.7	2.4
after 24 hours	0	1	1	0.7	0	0	0	0	0.7
after 48 hours	0	0	0	0	0	0	0	0	0.0
after 72 hours	0	0	0	0	0	0	0	0	0.0
	Erythema				**Oedema**				-
mean score 24 – 72 hours	**0**	**0.3**	**0.3**	**-**	**0**	**0**	**0**	**-**	-

[1]Primary Dermal Irritation Index reported in the study (sum of erythema + oedema scores).

Based on the scores for erythema and oedema found in the *in vivo* study and considering the CLP criteria, the DS did not propose a classification for skin corrosion/irritation.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

According to CLP Regulation Annex I, Table 3.2.2, a substance should be classified for skin irritation Category 2 in the cases where:

(1) *Mean value of ≥ 2,3 - ≤ 4,0 for erythema/eschar or for oedema in at least 2 of 3 tested animals from gradings at 24, 48 and 72 hours after patch removal or, if reactions are delayed, from grades on 3 consecutive days after the onset of skin reactions; or*

(2) *Inflammation that persists to the end of the observation period normally 14 days in at least 2 animals, particularly taking into account alopecia (limited area), hyperkeratosis, hyperplasia, and scaling reactions; or*

(3) *In some cases where there is pronounced variability of response among animals, with very definite positive effects related to chemical exposure in a single animal but less than the criteria above.*

The individual erythema scores in the 3 animals were 0, 0.3 and 0.3, and the oedema scores were 0, 0, 0, respectively". All mild skin irritation effects were fully reversible after 48 or 72 hours. Therefore, the CLP criteria for skin corrosion/irritation are not met, and RAC agrees with the DS's proposal that **no classification for skin corrosion/irritation is warranted**.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The DS provided one study on eye damage/irritation performed with New Zealand White rabbits according to OECD TG 405 (CA 5.2.5/01, B.6.2.5, study 1, 2012). 0.1 mL of the aqueous extract from the germinated seeds of sweet *Lupinus albus* was administered to three female rabbits as single instillation in conjunctival sac (see table below). Corneal opacity was observed in all three animals at 1h, in 2 animals at 24h and in 1 animal at 48h, thereafter no corneal opacity was observed. No iritis was observed in any of the animals at any time point. Conjunctival redness was observed in all three animals at 1h, 24h, 48h and 72h, but was no longer observed after 4 days. Conjunctival chemosis was observed in all three animals at 1h and 24h and in two animals at 48h; all animals were free of chemosis by 72h.

***Table**: Eye irritation scores according to the Draize scheme – unwashed eye*

Time / Rabbit No.	Cornea (opacity/area)			Iris (value/area)			Conjunctiva-					
							redness			chemosis / discharge		
	1	2	3	1	2	3	1	2	3	1	2	3
1 hour	1/2	1/2	1/1	0/0	0/0	0/0	2	2	2	3/2	3/3	2/2
24 hours	1/1	1/1	0/4	0/0	0/0	0/0	2	2	2	1/2	1/2	1/2
48 hours	1/1	0/4	0/4	0/0	0/0	0/0	1	2	1	1/1	1/1	0/0
72 hours	0/4	0/4	0/4	0/0	0/0	0/0	1	1	1	0/1	0/1	0/0
4 d	0/4	0/4	0/4	0/0	0/0	0/0	0	0	0	0/0	0/0	0/0
7 d	0/4	0/4	0/4	0/0	0/0	0/0	0	0	0	0/0	0/0	0/0
means scores 24-72 hours	**0.7**	**0.3**	**0**	**0**	**0**	**0**	**1.3**	**1.7**	**1.3**	**0.7**	**0.7**	**0.3**

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

According to CLP Regulation Annex I, Table 3.3.1, a substance should be classified in Category 1 (serious eye damage) if:

a) in at least one animal effects on the cornea, iris or conjunctiva that are not expected to reverse or have not fully reversed within an observation period of normally 21 days; and/or

b) in at least 2 of 3 tested animals, a positive response of
i) corneal opacity ⩾ 3 and/or
ii) iritis > 1,5

calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material.

In addition, a substance should be classified in Category 2 (eye irritation) if it produces in at least 2 of 3 tested animals, a positive response of (CLP Annex I, Table 3.3.2):

a) corneal opacity ⩾ 1, and/or
b) iritis ⩾ 1, and/or
c) conjunctival redness ⩾ 2, and/or
d) conjunctival oedema (chemosis) ⩾ 2

calculated as the mean scores following grading at 24, 48 and 72 hours after instillation of the test material, and which is fully reverses within an observation period of 21 days.

RAC notes that the eye effects were reversible in all animals, and no corneal opacity score of 3 or higher was observed and no iritis was observed in any of the animals. Therefore, the criteria for Cat. 1 classification are not met. Furthermore, corneal opacity mean scores over the 24-72-hour period were 0.7, 0.3 and 0 for the three animals thus below the classification threshold of 1. Iritis was not observed in any of the animals. Conjunctival redness mean scores over the 24-72-hour period were 1.3, 1.7, 1.3 for the three animals, again below the classification threshold of 2. In addition, conjunctival chemosis mean scores over the 24-72-hour period were 0.7, 0.7 and 0.3 for the three animals, thus below the classification threshold of 2 as well. Therefore, criteria for Cat. 2 classification are not met.

RAC agrees with the DS's proposal of **no classification for eye damage/irritation**.

RAC evaluation of skin sensitisation

Summary of the Dossier Submitter's proposal

The DS provided one study on skin sensitisation carried out with Hartley albino Guinea pigs according to OECD TG 406 method (Buehler assay) (CA 5.2.6/01, B.6.2.6, study 1, 2012). Four male animals have been used for pre-test by application of 0.4 mL of the aqueous extract from the germinated seeds of sweet *Lupinus albus* (25 %, 50 %, 75 % and 100 %) by an occlusive manner for 6 hours to determine the highest non-irritating concentration. Only at doses of 75 and 100 %, 2 out of 4 animals exhibited very faint skin erythema (score of 0.5). It was concluded that the highest non irritation concentration is 100 % used for further investigations both for the induction and challenge phases.

Twenty male animals were used for the main Buehler assay. Once each week for 3 weeks, 0.4 mL of the undiluted test article was applied to the left side of each test animal for 6 hours. Approximately 24 and 48 hours after each induction application readings of local reactions were made. Twenty-seven days after the first induction dose, 0.4 mL of the undiluted test article was applied on the right side of each animal as a challenge dose. These sites were evaluated for a sensitisation response approximately 24 and 48 hours after the challenge application. In addition, 10 male animals from a control group were treated with undiluted test article at challenge phase only.

Very mild to mild erythema (score 0.5-1) was noted for all test sites during the induction phase. In addition, very faint erythema (0.5) was noted for 12 out of the 20 test sites at 24 hours after challenge. Irritation cleared from all affected sites by 48h. Under the conditions of the study performed, the test substance did not show evidence of skin sensitisation.

In addition, the DS mentioned a position paper on the dermal penetration of BLAD – a polypeptide, the main component of the aqueous extract from the germinated seeds of sweet *Lupinus albus* (Gledhill, 2019). It is demonstrated that polypeptides such an insulin are not absorbable via the dermal route without significant measures to disrupt or bypass the stratum corneum. Insulin is a polypeptide consisting of 51 amino acid residues, so is a smaller molecule than BLAD and is thus considered to provide a worse-case scenario with respect to dermal absorption. Therefore, by analogy with a polypeptide such as insulin, it is reasonable to conclude that under normal use conditions BLAD will not reach the epidermis or dermis layers within the skin.

Comments received during consultation

One MSCA supported no classification for this hazard class. One comment is received during consultation from Switzerland`s MSCA on the supplementary information provided by the DS concerning food allergy considerations caused by BLAD in general as the BLAD is present in different nuts and berries. RAC remarks that food allergenicity issues are not considered for skin sensitization assessment.

Assessment and comparison with the classification criteria

According to CLP Regulation (Annex I, 3.4.2.2.1), substances shall be classified as skin sensitisers (Cat. 1) where data are not sufficient for sub-categorisation, in accordance with the criteria in Table 3.4.2 therein:

a) if there is evidence in humans that the substance can lead to sensitisation by skin contact in a substantial number of persons; or

b) if there are positive results from an appropriate animal test.

Regarding the Buehler guinea pig assay, two sub-categories can be discriminated (Table 3.4.3 and Table 3.4.4):

a) Cat. 1A: *$\geqslant$ 15 % responding at $\leqslant$ 0.2 % topical induction dose; or $\geqslant$ 60 % responding at > 0.2 % to $\leqslant$ 20 % topical induction dose,*

b) Cat. 1B: *$\geqslant$ 15 % to < 60 % responding at > 0.2 % to $\leqslant$ 20 % topical induction dose; or $\geqslant$ 15 % responding at > 20 % topical induction dose.*

RAC notes that in the study conducted with the aqueous extract from the germinated seeds of sweet *Lupinus albus* only a very mild erythema (score 0.5) was observed at challenge phase after 24 hours which was resolved in all animals after 48 hours. This effect can be regarded as indicative of irritation, not of skin sensitisation. Therefore, RAC agrees with the DS's proposal of **no classification for skin sensitisation**.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier Submitter's proposal

The DS provided two studies on repeated exposure in relation to specific target organ toxicity carried out with Charles River Laboratories Wistar Hannover (CRL:WI(Han)) rats.

In the 90-day oral study performed according to OECD TG 408 method aqueous extract from the germinated seeds of sweet *Lupinus albus* was administered to 10 per sex/dose test animals by oral gavage applying the following doses: 0, 250, 500, 1000 mg/kg bw/day (CA 5.3.2/01, B.6.3.2 Study 1, 2016). No mortality, clinical signs, effects on body weight or body weight gain, food consumption, haematology or clinical chemistry were observed. No treatment-related effects have been revealed. There were no effects on organ weights or gross pathology as well. One female in the high dose group had minimal to slight bilateral symmetrical neutrophil vacuolation in the grey matter. As this effect was not observed in any of the other animals and no neurological findings were observed, this was considered an incidental finding. Nevertheless, based on a precautionary approach, the NOAEL was set at 500 mg/kg bw/day based on the vacuolation in brain and spinal cord observed in one female at the high dose.

In a 22-day dermal toxicity study performed according to OECD TG 410 method aqueous extract from the germinated seeds of sweet *Lupinus albus* was administered to 5 per sex/dose test animals in a non-occlusive manner (the test article was spread as uniformly as possible over the clipped dermal test site for 6 hours exposure daily) applying the following doses: 0, 100, 300 and 1000 mg/kg bw/day (CA 5.3.3/01, B.6.3.3 Study 1, 2015). No mortality, clinical signs, effects on body weight or body weight gain, food consumption, haematology or clinical chemistry were observed. The only effect on organ weight observed was an increased adrenal weight in males. The adrenal weights (adjusted for terminal body weight) increased by 16 %, 18 % and 38 % for doses of 100, 300 and 1000 mg/kg bw/day, respectively. No histopathological effects in the adrenals were observed. Minimal to slight hyperkeratosis of the treated skin was observed in males and females of the high dose group of 1000 mg/kg bw/day, characterised by a minor increase in thickness of the epidermis with increased keratohyalin granules. In the kidney hyaline droplets were increased in males at 1000 mg/kg bw/day, characterised by eosinophilic cytoplasmic inclusions in the proximal tubular epithelial cells. This effect was not observed in females. Hyaline droplets appear as eosinophilic cytoplasmic inclusions in the proximal tubular epithelial cells and are a common background finding in the kidney of male rats. They generally represent accumulations of α2u-globulin, a naturally occurring male rat protein, and it is a common response of the male rat to xenobiotics. The DS considered that the increase in hyaline droplets in kidneys in high dose males is not relevant for humans and the increase in adrenal weight in top dose males was not significant, within historical control data and not accompanied by histopathological findings. Nevertheless, the NOAEL was set at 300 mg/kg bw/day based on increased adrenal weight when adjusted for terminal body weight.

No classification for STOT RE was proposed by the DS.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

According to CLP Regulation, Annex I, 3.9.2.1, substances are classified as specific target organ toxicants following repeated exposure by the use of expert judgement on the basis of the weight

of all evidence available, including the use of recommended guidance values which take into account the duration of exposure and the dose/concentration which produced the effect(s). The guidance values for a 90-day study are:

a) for Cat. 1 classification (CLP Regulation, Table 3.9.2): Oral (rat): C ≤ 10 mg/kg bw/day
b) for Cat. 2 classification (CLP Regulation, Table 3.9.3): Oral (rat): 10 < C ≤ 100 mg/kg bw/day

while the adjusted guidance values for a 28-day study (CLP Regulation, Annex I, 3.9.2.9.5; CLP Guidance Table 3.16) are:

a) for Cat. 1 classification: Dermal (rat/rabbit): C ≤ 60 mg/kg bw/day
b) for Cat. 2 classification: Dermal (rat/rabbit): 60 < C ≤ 600 mg/kg bw/day.

RAC notes that, in the 90-day oral toxicity study, the only possible treatment related observed effect was vacuolation in the brain and spinal cord in one female at the highest dose of 1000 mg/kg bw/day which is above the threshold for classification, consequently, this finding does not justify classification as STOT RE.

RAC also considers that, in the 22-day dermal toxicity study, the only effect observed was increased adrenal weight at the highest dose of 1000 mg/kg bw/day without any histopathological findings. The adjusted guidance values for the 22-day test is 76 < C ≤ 762 mg/kg bw/day, which is below the effective dose detected, and therefore this finding does not justify classification as STOT RE.

In conclusion, RAC agrees with the DS's proposal for **no classification for STOT RE**.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier Submitter's proposal

The DS provided three *in vitro* studies and one *in vivo* study on mutagenicity/genotoxicity of aqueous extract from the germinated seeds of sweet *Lupinus albus*. The *in vitro* studies included:

a) Ames assay according to OECD TG 471 method with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 as well as with *E. coli* strain WP2uvrA (CA 5.4.1.1/01, B.6.4.1, study 1, 2016). The concentrations tested were up to 5000 µg/plate both with and without S9 metabolism activation system. In addition, positive controls by means of 2-nitrofluorene, 4-nitroquinoline-1-oxide, N-methyl-N'-nitro-N-nitroguanidine, ICR-191 and 2-aminoanthracene have been accomplished.

The test substance did not increase revertant colony numbers either with or without S9.

b) Mammalian cell gene mutation test according to OECD TG 476 method with mouse lymphoma cells (L5178Y) (CA 5.4.1.2/01, B.6.4.1, study 2, 2015). The concentrations tested were up to 5000 µg/mL without S9 activation system and up to 2500 µg/mL with S9. In addition, positive controls by means of methyl methane sulphonate and Benzo[a]pyrene were carried out. Preliminary cytotoxicity assay was conducted as well.

Without S9, the test substance did not induce mutation at the tk locus of lymphoma cells after 3 hours treatment when tested up to either a precipitating or toxic concentration. With S9, the test substance induced mutation at the tk locus after 3 hours treatment when tested up to toxic concentrations.

c) Micronucleus assay according to OECD TG 487 method with human peripheral blood lymphocytes (CA 5.4.1.3/01, B.6.4.1, study 3, 2015). The concentrations tested were up to 2000 µg/mL both with and without S9 metabolism activation system. In addition, positive controls by means of mitomycin C and vinblastine have been conducted. Preliminary cytotoxicity assay was conducted as well.

The test substance did not induce micronuclei in cultured human peripheral blood lymphocytes during treatment for 3 hours and following 21 hours of recovery as well as for 24 hours followed by 24 hours of recovery in the absence of S9. In addition, the test substance did not induce micronuclei during treatment for 3 hours followed by 21 hours of recovery in the presence of S9.

The *in vivo* Comet assay study according to OECD TG 489 method with Wistar Hannover rats covered the following doses of aqueous extract from the germinated seeds of sweet *Lupinus albus*: 0, 500, 1000, 2000 mg/kg bw administered by oral gavage to 5 male rats per dose (CA 5.4.2.1/01, B.6.4.2, Study 1, 2015). Two applied doses were separated by 21 hours. In addition, positive controls by ethyl methane sulphonate was conducted.

The test substance did not induce DNA damage in the stomach of male rats following dosing at 0 and 21 hours with harvesting of the stomach tissue 3 hours later. No clinical signs, effect on body weight or other test-article related toxicity was observed. It shall be remarked that stomach (first site of contact) was chosen as a target organ taking into account that the main component of the test substance is a protein which will be broken down and consumed by normal catabolic processes in the stomach.

Based on all of the studies available, the DS did not suggest classification for germ cell mutagenicity.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

According to CLP Regulation, Table 3.5.1, classification in Category 2 for germ cell mutagenicity is based on:

- *positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:*
  - *somatic cell mutagenicity tests in vivo, in mammals; or*
  - *other in vivo somatic cell genotoxicity tests which are supported by positive results from in vitro mutagenicity assays*.

RAC notes that the aqueous extract from the germinated seeds of sweet *Lupinus albus* gave negative test result in an Ames test and negative test result in an *in vitro* micronucleus assay with human lymphocytes. The *in vitro* mammalian cell gene mutation assay (with S9 only) was positive but the *in vivo* Comet assay was negative.

RAC considers that the DS's conclusion of no classification is justified and supports **no classification for germ cell mutagenicity**.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

The DS stated that long-term toxicity and carcinogenicity studies have not been conducted and are not considered necessary. Aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20 % of BLAD as the lead component. BLAD is a naturally occurring polypeptide formed during day four to twelve of the germination process of sweet *Lupinus albus*. BLAD is used in human and animal nutrition as a food and feed component. It has a non-toxic mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure) and is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down entering the amino acid pool and will be consumed by normal catabolic processes.

The DS indicated that the 90-day oral study was completely negative, and the aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered to be non-genotoxic, and there are no possible mechanisms for carcinogenicity foreseen.

In addition, there is no evidence that proteins similar to BLAD are associated with an increased incidence of cancer. Based on these considerations it can be concluded that the lead component is unlikely to be considered a carcinogen.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

RAC agrees that there is no evidence that aqueous extract from the germinated seeds of sweet *Lupinus albus* could be a carcinogen. The main component BLAD is a naturally occurring polypeptide which is rapidly biodegradable. In agreement with the DS, RAC notes that the 90-day oral study was negative, and the substance is not classified for germ cell mutagenicity. Therefore, RAC supports **no classification for carcinogenicity**.

RAC evaluation of reproductive toxicity

Summary of the Dossier Submitter's proposal

The DS indicated that reproductive toxicity studies (including developmental studies) have not been conducted and are considered not necessary. Aqueous extract from the germinated seeds of sweet *Lupinus albus* contains 20 % of BLAD as the lead component. BLAD is a naturally occurring polypeptide formed during day four to twelve of the germination process of sweet *Lupinus albus*. BLAD is used in human and animal nutrition as a food and feed component. It has a non-toxic mode of action which is specific to fungi only (BLAD binds to chitin and chitosan which weakens the cell wall structure) and is rapidly biodegradable. It is known to be susceptible to proteolytic degradation and the protein will be broken down entering the amino acid pool and will be consumed by normal catabolic processes.

In addition, there is no evidence that proteins similar to BLAD are associated with reproductive toxicity. Based on these considerations it can be concluded that the lead component is unlikely

to be considered as a reproductive toxicant, and classification for reproductive toxicity is not proposed.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

RAC agrees that there is no evidence that aqueous extract from the germinated seeds of sweet *Lupinus albus* could be a reproductive toxicant. The main component BLAD is a naturally occurring polypeptide which is rapidly biodegradable. Therefore, RAC supports **no classification for reproductive toxicity.**

RAC evaluation of aspiration toxicity

Summary of the Dossier Submitter's proposal

The DS provided one study characterising the aspiration toxicity properties of aqueous extract from the germinated seeds of sweet *Lupinus albus* performed according to OECD TG 114 method for assessment of kinematic viscosity (Wo, 2012b). The kinematic viscosity detected at 20 °C was 765.932 mm^2/s and at 40 °C – 230.181 mm^2/s.

No classification for aspiration toxicity was proposed by the DS.

Comments received during consultation

One MSCA supported no classification for this hazard class.

Assessment and comparison with the classification criteria

According to CLP Regulation, substances shall be classified for aspiration toxicity if the kinematic viscosity of the substance is 20.5 mm^2/s (measured at 40 °C) or lower. RAC notes that the measured values of kinematic viscosity of aqueous extract from the germinated seeds of sweet *Lupinus albus* at 40 °C is 230.181 mm^2/s, and the related criterion is not met.

RAC supports **no classification of aqueous extract from the germinated seeds of sweet *Lupinus albus* for aspiration toxicity**.

ENVIRONMENTAL HAZARD EVALUATION

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

The aqueous extract from the germinated seeds of sweet *Lupinus albus* (Sweet Lupin (seeds)) is an active substance in plant protection products (fungicide) that can be used on food and non-food crops. The aqueous extract from the germinated seeds of sweet *Lupinus albus* is currently

not included in Annex VI of CLP Regulation. The DS proposed not to classify the aqueous extract from the germinated seeds of sweet *Lupinus albus* as hazardous to the aquatic environment.

Degradation

There are three ready biodegradability studies available.

In the first study (Brunswik-Titze, 2015), the biodegradation of the BLAD protein (major constituent of the Aqueous extract from the germinated seeds of sweet *Lupinus albus*) was determined with CO_2-evolution test (OECD TG 301B) over 28 days. Degradation of BLAD protein reached 64.7 % after 7 days and therefore the criteria of 60 % degradation within a 14-day window is met. The aqueous extract from the germinated seeds of sweet *Lupinus albus* is therefore considered as readily biodegradable under the test conditions.

In the second study (Carreira, 2014), which was performed in line with OECD TG 301D - closed bottle test, the degradation of BLAD protein was determined using a specific analytical technique (sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)) for detection of the BLAD protein. The study was conducted with two different inoculants from two separate waste treatment plants with two different concentrations of BLAD protein (100 and 200 mg/L) and two concentrations of inoculum (0.5 and 5 mL/L). In the study the degradation of the BLAD protein was rapid with very little or no BLAD protein remaining after 14 days. The degradation of the lower concentration (100 mg/L) of BLAD protein was greater than 90 % in 7 days, while the degradation was a little slower with higher concentration (200 mg/L) of BLAD protein, but the protein was completely degraded after 14 days (> 98 %) in all tests expect in one (lower concentration of BLAD protein and inoculum concentration of 0.5 mL/L) where 3 % was remaining after 14 days.

The third study (Dengler, 2010), carried out using closed bottle study (OECD TG 301D), investigated the degradation of Aqueous extract from the germinated seeds of sweet *Lupinus albus* (contain 20 % of BLAD protein) over 28 days. Degradation of aqueous extract reached 81.4 % after 14 days and 91.7 % after 28 days, therefore the aqueous extract from the germinated seeds of sweet *Lupinus albus* was considered as readily biodegradable under the test conditions.

Based on these studies the DS concluded that Aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered rapidly degradable.

Bioaccumulation

Bioaccumulation in fish and other organisms is not expected.

Sweet lupin seeds extract is a complex mixture and consists of numerous components, none of which are isolated during the preparation of the product. It is therefore not possible to derive a Log K_{ow} for Aqueous extract from the germinated seeds of sweet *Lupinus albus* as a whole. Furthermore, estimates for many of these components would be very difficult as they are broad classes of compounds and defining their structure would be problematic. Therefore, the assessment of the potential for bioaccumulation is focused on the known constituents only.

The lead component, BLAD protein, is a naturally occurring seed storage protein in germinated sweet lupines. It is a 210 kDa gylco-oligomer which is mainly composed by a 20 kDa protein of β-conglutin or characterised as a fragment of the amino acid sequence of β-conglutin, therefore, there is no specific molecular or structural formula. As such a Log K_{ow} cannot be estimated using QSAR modelling. A theoretical Log K_{ow} value was calculated indicating a value of 4.5 for the lead component (BLAD protein). Similarly for a component that constitutes 0.04 % of Aqueous extract from the germinated seeds of sweet *Lupinus albus* a Log K_{ow} > 4 was reported. For other two components, theoretical Log K_{ow} values of less than 4 were calculated.

The main component of Aqueous extract from the germinated seeds of sweet *Lupinus albus* is a BLAD protein that will be broken down in the digestive track of animals, entering the amino acid pool and consumed under catabolic processes and is unlikely to bioaccumulate. The other constituent present in the product with a Log K_{ow} > 4 has a weight concentration of 0.04 %. As this percentage is ≤ 0.1 %, it is considered as a marginal quantity for the product to have a potential to bioaccumulate.

Based on the presented data, the DS concluded that Aqueous extract from the germinated seeds of sweet *Lupinus albus* has a low potential for bioaccumulation.

Aquatic toxicity

Reliable aquatic toxicity data for Aqueous extract from the germinated seeds of sweet *Lupinus albus* are available for all three trophic levels (except chronic toxicity to fish) in the CLH report. A summary of the relevant information on aquatic toxicity is provided in the following table (the key endpoints used in hazard classification are highlighted in bold).

***Table**: Summary of reliable information on acute and chronic aquatic toxicity*

Method/Exposure/Test material	Species	Endpoint	Toxicity value (mg/L)	Reference
Acute aquatic toxicity				
OECD TG 203, GLP, semi-static system **Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD protein content 20.0 % w/w**	*Oncorhynchus mykiss*	96h LC_{50}	> 100 n > 50 n, c	Anonymous, 2011
OECD TG 202, GLP, semi-static system **Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD protein content 20.0 % w/w**	*Daphnia magna*	48h EC_{50}	159.32 n 79.66 n, c	Weber, 2011
OECD TG 202 (2006), GLP, static-renewal system **Aqueous extract from the germinated seeds of sweet *Lupinus albus,* 100 % w/w**	*Daphnia magna*	48h EC_{50}	> 75 gm	Gerke and Schneider, 2019
OECD TG 201, GLP, static test system **Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD protein content 20.0 % w/w**	*Desmodesmus subspicatus*	72h E_yC_{50}	28.7 n 14.35 n, c	Falk, 2011
OECD TG 201, GLP, static test system **Aqueous extract from the germinated seeds of sweet *Lupinus albus* (100 % w/w), BLAD protein content 21.0 % w/w**	*Raphidocelis subcapitata*	72h E_rC_{50} 72h E_yC_{50}	**51** gm 12 gm	Arnie *et al.*, 2019
Chronic aquatic toxicity				
OECD TG 201, static test system **Aqueous extract from the germinated seeds of sweet *Lupinus albus*, BLAD protein content 20.0 % w/w**	*Desmodesmus subspicatus*	72h NOEC	11.1 n 5.5 n, c	Falk, 2011

OECD TG 201, static test system **Aqueous extract from the germinated seeds of sweet *Lupinus albus* (100 % w/w), BLAD protein content 21.0 % w/w**	*Raphidocelis subcapitata*	72h E_rC_{10}	7.5 gm	Arnie *et al.*, 2019
OECD TG 211, GLP, static-renewal system **Aqueous extract from the germinated seeds of sweet *Lupinus albus*, 100 % w/w**	*Daphnia magna*	21d EC_{10} 21d NOEC	**> 2.7** gm 2.7 gm	Gerke and Scheneider, 2019

Note: n – nominal concentration; gm - geometric mean measured concentration; c – corrected value (considering possible degradation of the aqueous extract from the germinated seeds of sweet *Lupinus albus*);

In the CLH report, there was also a consideration of possible degradation of the aqueous extract from the germinated seeds of sweet *Lupinus albus* during the tests performed with fish, *Daphnia* and algae. Analytical verification of the test solutions was not conducted in three studies, *O. mykiss* study (Anonymous, 2011), *D. magna* study (Weber, 2011) and *D. subspicatus* study (Falk, 2011). Due to the lack of analytical verification throughout the tests it was not known exactly what the measured concentrations were at the end of the 72-hour test period in the algal study or at the end of the 24-hour renewal periods in fish and *Daphnia* studies. The DS has addressed this uncertainty with the results of the available ready biodegradation study (OECD TG 301B) (Brunswik-Titze, 2015). The results of the study demonstrated that at the first measurement timepoint on Day 4 the total biodegradation ranged between 47.9 - 50.8 % in the three test replicates, with a mean value of 49.2 %. Thus, it can be concluded that the degradation of BLAD protein was approximately 50 % after 4 days. This assumption of 50 % degradation over 4 days could therefore be used to predict the stability of the test material over the course of each test. Application of the 50 % degradation value to the results of each test could allow for an estimation of the likely measured concentrations in the test media after 4 days. Nevertheless, it should be noted that this was a very conservative assumption because in fish and *Daphnia* studies the test media was renewed every 24 hours therefore it is a highly conservative approach to assume that all of this 50 % degradation would have occurred in the first 24 hours when, in all likelihood, the actual degradation over 24-hours would have been less. Likewise, in the algal study, the test was only 72-hours in duration and therefore this test period is also covered by the 50 % degradation value over 4 days. The DS proposed to adjust the current endpoints from the three aquatic studies by a factor of 2 to account for an approximate 50 % degradation over time. Again, this was very conservative as it did not account for the fact that nominal concentrations were likely to have been achieved at the start of each test or renewal period for each study, thereby leading to mean concentrations much greater than 50 % of the nominal value. This means that the reported endpoints (effect concentrations) as obtained from the aquatic studies were lower than the actual a.s. concentrations in the study set-up. Thus, any uncertainty in extrapolating the measured result from the biodegradation study to the fish, *Daphnia* and algal studies was considered to be covered by this correction factor of 2. The current aquatic toxicity endpoints for Aqueous extract from the germinated seeds of sweet *Lupinus albus* as well as the corrected endpoints for Anonymous (2011), Weber (2011) and Falk (2011) are presented in the table above.

Acute Aquatic toxicity

Reliable aquatic acute toxicity data were available for all three trophic levels (fish, invertebrates and algae). The lowest endpoint for fish was the nominal 96h LC_{50} value of > 50 mg/L for *Oncorhynchus mykiss*, for invertebrates the mean measured 48h EC_{50} value of > 75 mg/L for *Daphnia magna* and for algae the mean measured 72h E_rC_{50} value of 51 mg/L for *Raphidocelis subcapitata*. The lowest acute toxicity value of 51 mg/L was above classification threshold value

of 1 mg/L, therefore no classification of Aqueous extract from the germinated seeds of sweet *Lupinus albus* is warranted.

Chronic Aquatic toxicity

Reliable aquatic chronic toxicity data were available for invertebrates and algae, while data for fish were lacking. From the available aquatic toxicity data, invertebrates are the most sensitive trophic group. The lowest chronic endpoint was the mean measured 21d EC_{10} of > 2.7 mg/L, reported for *Daphnia magna*. Based on aquatic chronic toxicity data for *Daphnia* and considering the aqueous extract from the germinated seeds of sweet *Lupinus albus* as rapidly degradable and with a low potential for bioaccumulation, the DS concluded that Aqueous extract from the germinated seeds of sweet *Lupinus albus* does not meet the classification criteria for aquatic chronic hazard.

Classification on the basis of the acute data for fish as surrogate is not required because the aqueous extract from the germinated seeds of sweet *Lupinus albus* is considered to be rapidly biodegradable and to have a low potential to bioaccumulate. For this reason, also the classification as Aquatic chronic 4 is not required.

Comments received during consultation

A comment was received from a MSCA, which agreed to the proposal that the aqueous extract from the germinated seeds of sweet *Lupinus albus* should not be classified as hazardous to the aquatic environment.

Assessment and comparison with the classification criteria

Degradation

RAC agrees with the DS to consider the aqueous extract from the germinated seeds of sweet *Lupinus albus* as rapidly degradable based on:

- 91.7 % degradation of BLAD protein in OECD TG 301B Ready biodegradability test after 28 days,
- Complete degradation (except in one sample; 3 % remained) of BLAD protein in OECD TG 301D Closed bottle test after 14 days, with suggested DT_{50} < 2 days and
- 91.7 % degradation of Aqueous extract from the germinated seeds of sweet *Lupinus albus* (contain 20 % of BLAD protein) in an OECD TG 301D Ready biodegradability test after 28 days.

Bioaccumulation

RAC agrees with the DS to consider the aqueous extract from the germinated seeds of sweet *Lupinus albus* having a low potential for bioaccumulation based on:

- BLAD protein, the main component of the aqueous extract from the germinated seeds of sweet *Lupinus albus* has a theoretical Log K_{ow} value of 4.5. Nevertheless, the BLAD protein will be broken down in the digestive track of animals, entering the amino acid pool and consumed into normal catabolic processes, therefore it is considered unlikely to bioaccumulate.
- Constituent that constitutes 0.04 % of the aqueous extract from the germinated seeds of sweet *Lupinus albus* has a theoretical Log K_{ow} > 4. However, as the constituent is present in the concentration ≤ 0.1 % it is considered as a marginal quantity for the aqueous extract from the germinated seeds of sweet *Lupinus albus* to have a potential to bioaccumulate. This is in line with ECHA guidance (Part C: PBT/vPvB Assessment, version

3.0, June 2017) where only the constituents present in concentrations ≥0.1 % w/w in the aqueous extract from the germinated seeds of sweet *Lupinus albus* must be considered for bioaccumulation assessment.
- Two other constituents of the aqueous extract from the germinated seeds of sweet *Lupinus albus* have a theoretical Log K_{ow} < 4.

Acute Aquatic toxicity

In case of the aqueous extract from the germinated seeds of sweet *Lupinus albus*, reliable acute toxicity data are available for all three trophic levels. Algae are the most sensitive group, and the lowest toxicity value is a mean measured 72h E_rC_{50} of 51 mg/L for *Raphidocelis subcapitata*. RAC notes that all EC_{50}s/LC_{50}s for fish, invertebrates and algae (see table above) are above the threshold value of 1 mg/L therefore the aqueous extract from the germinated seeds of sweet *Lupinus albus* does not meet the criteria for classification for acute aquatic hazard.

Chronic Aquatic toxicity

Reliable chronic toxicity data on the aqueous extract from the germinated seeds of sweet *Lupinus albus* are available for two trophic levels: invertebrates and algae. Data for fish are lacking. The lowest chronic effect value is the mean measured 21d EC_{10} of > 2.7 mg/L from a test with the crustacea *Daphnia magna*. RAC notes that all available information shows no adverse effects of the aqueous extract from the germinated seeds of sweet *Lupinus albus* to aquatic organisms (see table above) below the threshold value of 1 mg/L. As discussed previously, the aqueous extract from the germinated seeds of sweet *Lupinus albus* is rapidly degradable and has low potential to bioaccumulation. Based on this, RAC concludes that the aqueous extract from the germinated seeds of sweet *Lupinus albus* does not fulfil the criteria for chronic hazard classification.

RAC is of the opinion that in line with CLP guidance section 4.1.3.3 and Table 4.1.0(b)(iii) the criteria for using the acute toxicity data for fish as surrogate are not fulfilled for the aqueous extract from the germinated seeds of sweet *Lupinus albus* as it is considered rapidly degradable and does not fulfil the criteria for bioaccumulation.

Based on the aqueous extract from the germinated seeds of sweet *Lupinus albus* being rapidly degradable, having a low potential for bioaccumulation, and there being no concerns regarding aquatic toxicity, RAC also agrees that classification of the aqueous extract from the germinated seeds of sweet *Lupinus albus* as Aquatic Chronic 4 is also not warranted.

Overall, RAC agrees to the DS's proposal **not to classify the aqueous extract from the germinated seeds of sweet *Lupinus albus* as hazardous to aquatic environment.**

ANNEXES:

Annex 1 The Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the DS; the evaluation performed by RAC is contained in 'RAC boxes'.

Annex 2 Comments received on the CLH report, response to comments provided by the DS and RAC (excluding confidential information).