CONCLUSION ON PESTICIDE PEER REVIEW

Conclusion on the peer review of the pesticide risk assessment of the active substance fenugreek seed powder (FEN 560)[1]

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SUMMARY

Fenugreek seed powder (FEN 560) is a new active substance for which, in accordance with Article 6 (2) of Council Directive 91/414/EEC,[3] France received an application from Société Occitane de Fabrications et de Technologies (SOFT) for inclusion in Annex I to Directive 91/414/EEC. Complying with Article 6 of Directive 91/414/EEC, the completeness of the dossier was evaluated and confirmed by Commission Decision 2004/131/EC[4].

Following the agreement between the European Commission and the European Food Safety Authority (EFSA) for the EFSA to organise a peer review of those new active substances for which the decision on the completeness of the dossier had been published after June 2002, the designated rapporteur Member State France made the report of its initial evaluation of the dossier on fenugreek seed powder, hereafter referred to as the draft assessment report (DAR), available on 6 January 2005.

The peer review was initiated on 15 March 2005 by dispatching the DAR for consultation of the Member States and the applicant. Subsequently, the comments received on the DAR were examined by the rapporteur Member State in the reporting table and the need for additional data was agreed in an evaluation meeting in November 2005. The identified issues as well as further data made available by the applicant upon request were evaluated in a series of scientific meetings with Member State experts in September 2006, November 2006, December 2006 and May 2009.

A final consultation on the outcome of the experts' discussions took place during a written procedure with the Member States in October – November 2009 leading to the conclusions as laid down in this report.

This conclusion was reached on the basis of the evaluation of the representative uses as a plant activator on grapevines. Full details of the GAP can be found in the list of end points.

The representative formulated product for the evaluation was 'Stimulia', the applicant proposed that the formulation is a soluble powder (SP). The formulation was questioned during the peer review process and the formulation type remains unclear.

Methods of analysis for monitoring are not required due to the nature of this product. The majority of the physical-chemical properties data were considered to be not relevant for this product. It was

1 On request from the European Commission, Question No EFSA-Q-2009-00314, issued on 18 December 2009.

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3 OJ No L 230, 19.8.1991, p. 1. Directive as last amended by L 20, 22.1.2005, p.19

4 OJ No L 37, 10.2.2004, p.34

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agreed that this product should be specified as fenugreek seed powder 100 %. Marker components were selected for quality control purposes but concentration ranges for these components were not agreed. Also a specification limit for mycotoxins has not been agreed.

Sufficient analytical methods as well as methods and data relating to physical, chemical and technical properties are available to ensure that some quality control measurements of the plant protection product are possible. There are some storage stability data outstanding although it is noted that these are available in the 2009 addendum to Volume 3 B.2 but are not peer reviewed. A sprayability study for the formulation is needed as the product failed the wet sieve test. The infra-red spectrum for fenugreek seed powder is also a data requirement.

With regard to the mammalian toxicity assessment, most of the information was from the published literature. Tests performed in 2006 demonstrated the absence of skin or eye irritating properties, and no mutagenic effect was observed in an Ames test. The powder is of low acute oral toxicity in mice and rats. Published short-term rat studies of limited validity showed a no adverse effect level (NOAEL) of 2500 mg/kg bw/day. The available information did not address the concern regarding potential reproductive toxicity and hormonal activity. Therefore a reliable NOAEL for these effects could not be established and reference values could not be set. Consequently, the risk assessment for the operators, bystanders and workers cannot be concluded.

Limited experimental data are available on the fate of fenugreek seed powder when applied to grapevine. Fenugreek seed powder is not considered to act systemically in plants. It contains natural substances and it seems reasonable to assume that these substances are degraded according to the known metabolic pathways for plant matter. Therefore it was concluded that the pertinent residue on treated crops to be considered in a consumer risk assessment is fenugreek seed powder. It was agreed not to propose a residue definition for monitoring and MRL's for residues of fenugreek seed powder in grapevine.

The notified use on grapevine did not require the assessment of residues in following crops and in livestock.

No data on the amounts of fenugreek seed powder present on the crop at the time of harvest were submitted. Hence, estimates were made on the basis of the application rate, crop density and surface area assuming no wash-off or degradation occurred until harvest. On this basis, approximate values for consumer intake of fenugreek seed powder from treated grapes and leaves were estimated.

Since no toxicological reference values could be established for fenugreek seed powder, a comparison of estimated and acceptable daily intake for the consumer has not been not possible. There are also no data available to compare the estimated consumer intake following a pesticidal use of fenugreek seed powder to the amounts of fenugreek seed spice usually consumed in the human diet. The only parameters available for comparison are doses administered in medical practice, or as a dietary supplement; however these applications are always intended to serve a particular purpose, and such products usually carry a warning label. Though it was agreed that the consumer intake of fenugreek seed powder in terms of the use in grapevine is overestimated by the theoretical calculations, however it could not be agreed by all experts that a comparison to similar doses of fenugreek seed administered as medical products or dietary supplements was appropriate.

No experimental data on the fate and behaviour of fenugreek seed powder were submitted. The risk assessment was performed on basis of estimated worst case initial exposure and on basis of the following case elements presented by the applicant:

> 'The fenugreek seed powder contains natural substances present in most plants. It is reasonable to consider that the different natural substances are degraded in soil in accordance with the known metabolic ways in living matter and this degradation leads to simple components also present in the natural environment.
>
> 'It is also underlined that there is no report about pollution by fenugreek seeds near fenugreek crops: during its annual cycle, fenugreek seeds fall from the arborescence at the end of

maturation and are scattered on the surrounding ground. The natural substances present in the seeds integrate the natural vegetal cycle to be decomposed into simple components.

'Generally, the harvest of fenugreek seeds reaches 4000 kg per hectare. It is reasonable to consider that, in this case, the level of the seeds debris on the soil is higher than the amount of the plant protection product which falls on the soil after treatment of grapevine, in view of the utilized dose (1.5 kg per hectare).'

The risk to non-target organisms was expected to be low for the representative use evaluated.

Key words: **fenugreek seed powder, peer review, risk assessment, pesticide, plant activator, ellicitor**

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BACKGROUND

In accordance with Article 6 (2) of Council Directive 91/414/EEC France received an application from the Société Occitane de Fabrications et de Technologies (SOFT) for inclusion of the active substance fenugreek seed powder (FEN 560) in Annex I to Directive 91/414/EEC. Complying with Article 6 of Directive 91/414/EEC, the completeness of the dossier was evaluated and confirmed by Commission Decision 2004/131/EC[5].

Following the agreement between the European Commission and EFSA for EFSA to organise a peer review of those new active substances for which the completeness of the dossier had been officially confirmed after June 2002, the designated rapporteur Member State France submitted the report of its initial evaluation of the dossier on fenugreek seed powder, hereafter referred to as the draft assessment report (DAR), to the EPCO team at the Bundesamt für Verbraucherschutz und Lebensmittelsicherheit (BVL) in Braunschweig, Germany on 6 January 2005. The DAR was distributed for consultation to the Member States and the applicant on 15 March 2005.

The comments received on the DAR were evaluated and addressed by the rapporteur Member State in the reporting table. Based on this evaluation, representatives from Member States identified and agreed in an evaluation meeting on 29 November 2005 on data requirements to be addressed by the applicant as well as issues for further detailed discussion at expert level. A representative of the applicant was attending this meeting. The identified issues as well as further data made available by the applicant upon request were evaluated in a series of scientific meetings with Member State experts in September 2006, November 2006, December 2006 and May 2009. The reports of these meetings have been made available to the Member States electronically.

A final consultation on the outcome of the experts' discussions took place during a written procedure with the Member States in October – November 2009 leading to the conclusions as laid down in this report.

During the peer review of the DAR and the consultation of technical experts no critical issues were identified for consultation of the Scientific Panel on Plant Health, Plant Protection Products and their Residues (PPR).

Following the agreement between the European Commission and EFSA regarding the peer review of new active substances, this conclusion summarises the results of the peer review on the active substance and the representative formulation evaluated as finalised at the end of the examination period. A list of the relevant end points for the active substance as well as the formulation is provided in appendix A.

The documentation developed during the peer review was compiled as a peer review report (EFSA, 2009) comprising of the documents summarising and addressing the comments received on the initial evaluation provided in the rapporteur Member State's DAR:

- the comments received,
- the resulting reporting table (revision 1-1; 20 December 2005),

as well as the documents summarising the follow-up of the issues identified as not finalised at the end of the commenting period:

- the reports of the scientific expert consultation,
- the evaluation table (revision 4-1; 25 November 2009).

Given the importance of the DAR including its addendum (compiled version of November 2009 containing all individually submitted addenda) and the peer review report with respect to the examination of the active substance, both documents are considered respectively as background documents A and B to this conclusion.

5 OJ No L 37, 10.2.2004, p.34

THE ACTIVE SUBSTANCE AND THE FORMULATED PRODUCT

All the active components of this product have not been identified. However, it was agreed that this product should be specified as 100 % fenugreek seed powder without any additive and without any extraction; the seed being of human food grade quality.

The mode of action has not been fully elucidated. It has been proposed that this product acts as a plant activator, eliciting the plant's own immune response against pathogens.

The representative formulated product for the evaluation was 'Stimulia', the applicant proposed that the formulation is a soluble powder (SP). The formulation was questioned during the peer review process and the formulation type remains unclear.

The evaluated representative use is as a plant activator on grapevines. Full details of the GAP can be found in the list of end points (appendix A).

SPECIFIC CONCLUSIONS OF THE EVALUATION

1. Identity, physical/chemical/technical properties and methods of analysis

The minimum purity of the product is 100 % fenugreek seed powder without any additive and without any extraction; the seed being of human food grade quality. Three marker components were identified, namely trigonelline, 4-hydroxyisoleucine and total proteins. It was agreed that concentration ranges rather than maximum amounts should be given for these components and this information is outstanding. Furthermore, a supported specification for mycotoxins has not been provided. At the moment no FAO specification exists.

The assessment of the data package revealed no issues that need to be included as critical areas of concern with respect to the identity, physical, chemical and technical properties of fenugreek seed powder. However, the following data gaps were identified with respect to the identity, physical, chemical and technical properties:

- Shelf life study with analysis of the marker components and mycotoxins before and after storage as well as a sprayability study. It is noted that a storage study was included in the 2009 addendum to Volume 3 B.2 but it has not been peer reviewed.
- Infra-red spectrum for fenugreek seed powder.
- Structures for the specific marker compounds.

It should be noted that the marker compound 4-hydroxyisoleucine was found to be unstable under accelerated storage conditions.

The main data regarding the identity of fenugreek powder and its physical and chemical properties are given in appendix A.

Sufficient test methods and data relating to physical, chemical and technical properties are available. Also adequate analytical methods are available for the technical material and the representative formulation as well as for the determination of the marker compounds.

Therefore, enough data are available to ensure that some quality control measurements of the plant protection product are possible.

Methods of analysis for monitoring purposes are not required due to the nature of this product.

2. Mammalian toxicity

Fenugreek seed powder was discussed by the experts in mammalian toxicology in three meetings: PRAPeR 4 in September 2006, PRAPeR 9 in November 2006, and PRAPeR 69 in May 2009. The conclusions in this section are based on the DAR (France, 2004), the addenda to Volume 3 (August

2006, October 2006, October 2007, February 2009) and to Volume 4 (October 2006) from the final addendum to the DAR (France, 2009).

Fenugreek seeds contain a complex mixture of chemical substances for which three markers were proposed by the applicant: total proteins content, trigonelline and 4-hydroxyisoleucine . Among other constituents mentioned in the literature were the saponins/sapogenins (2 to 6 %), including diosgenin (a steroid compound, representing 39 % of the saponins, see also section 2.6).

Different test materials were used in the published acute and short-term toxicological studies available in the dossier (without identification/analysis of batches): debitterized fenugreek seed powder (defatted, therefore without saponins), lyophilised aqueous extract of fenugreek leaves, fenugreek seed flour. In the more recent studies performed in 2006 (skin/eye irritation, skin sensitisation and mutagenicity tests), the batch B1 of seed powder was used and its composition (according to the proposed use of markers) was given in the addendum 1 to Volume 4 (October 2006).

The equivalence of the toxicological batches with the manufactured material was discussed by the experts. Since the development of plants is dependent on many environmental parameters, the material will vary and the batches will therefore be different and not comparable. It was agreed that the batch B1 was representative of the proposed manufactured material. Additionally, the use of fenugreek seed powder as a condiment and food supplement in humans could be relevant information since this was performed with the entire seed or seed powder. Even though some toxic activity was shown to be linked to the content of saponins in the 90-day studies performed with debitterized seeds (without saponins) and seed flour (with saponins), the experts considered that the debitterized seeds used in the acute oral test were sufficiently representative (see also sections 2.2 and 2.3).

2.1. Absorption, distribution, excretion and metabolism (toxicokinetics)

In the DAR, no toxicokinetic data were available. This was not considered needed during the peer-review.

2.2. Acute toxicity

Results from the literature were provided in the DAR. The extracts of fenugreek leaves were not considered to be representative of the seed powder (representative product) and only the studies with debitterized fenugreek seed powder were taken into consideration. Even though this tested seed powder was defatted, it was considered unlikely that the presence of up to 6 % saponins in fenugreek seed powder could increase notably the acute toxicity of the active substance. Therefore the powder was agreed to be of low acute oral toxicity (indicative rat oral LD_{50} >5000 mg/kg bw; indicative mouse oral LD_{50} >2000 mg/kg bw), based on the available information.

In the addendum of August 2006, new valid skin/eye irritation and skin sensitization tests were presented. Based on the results, the experts agreed that fenugreek seed powder was neither skin or eye irritating, nor a skin sensitizer.

2.3. Short-term toxicity

The results of two published 90-day rat studies were presented in the DAR. In the first 90-day rat study, performed with debitterized fenugreek seed powder, no adverse effect was observed at the highest dose level of 5000 mg/kg bw/day. In the second 90-day rat study, performed with fenugreek seed flour, the NOAEL of 2500 mg/kg bw/day was based on decreased cholesterol in males (20 % decrease) and increased relative liver weight in females (14 % increase). Based on these two studies, the experts agreed that the repeat dose toxicity of fenugreek seed powder was probably linked to the saponins content since no adverse effects were observed after the administration of debitterized seed powder (without saponins). It was also highlighted that these studies had a limited validity since no raw data were available.

2.4. Genotoxicity

In the DAR, no genotoxicity data were available, but additional data from the literature were presented for some components of the fenugreek seed. A fenugreek seed extract (detailed composition not available but containing minimum 40 % 4-hydroxyisoleucine) was not mutagenic in a battery of genotoxicity tests. Trigonelline was negative in the Ames test and mouse lymphoma assay. Flavonoids were reported as anti-mutagenic compounds. No data on the potential genotoxic activity of the saponins/sapogenins were available.

In the addendum to Volume 3 of August 2006, the results of a new Ames test with fenugreek seed powder (batch B1) were reported and agreed by the experts as being negative.

2.5. Long-term toxicity and carcinogenicity

In the DAR, no data on long-term toxicity and carcinogenicity were available. Further details about historic uses were considered under the point 2.9.

2.6. Reproductive and developmental toxicity

In the DAR, no data on reproductive and developmental toxicity were available. However some concerns related to a potential for reproductive toxicity were raised based on the information available about the chemical constituents of fenugreek seed powder and on the use of fenugreek in traditional or alternative medicine.

The hormonal activity of the constituent diosgenin has been discussed by the experts. It has to be taken into account that diosgenin represents about 39% of the steroidal sapogenins, which account for up to 6% of the composition of fenugreek seeds (see addendum of August 2006 in France, 2009). Considering that not all the constituents of the fenugreek seeds had been identified, and that it was not apparent which one was the active ingredient, the experts concluded that further investigation of the hormonal activity of fenugreek seed powder was needed (data requirement), pending on the agreement of the technical specification.

The potential stimulating properties of uterine contraction of fenugreek seeds (and the risk to pregnant women) were also discussed by the experts. The concern originated from a publication on the therapeutic applications of fenugreek, mentioning an experiment performed on isolated guinea pig uterus by Abdo MS in 1969 (see addendum of October 2007 in France, 2009). No further details were available and conclusion could not be drawn.

In the addendum of October 2006, a more recent published study by Kassem on the potential antifertility effect of fenugreek seeds in rabbits was evaluated. This non-GLP and non-guideline compliant study had been performed with only one very high dose (300 000 ppm in the diet). No NOAEL could be determined since a toxic effect on testis was observed, as well as embryotoxicity and changes in ovary and uterus morphology. The minimum active dose was unknown.

The experts concluded that, in humans, a possible effect of fenugreek seeds on milk production and on uterine contraction cannot be excluded. Additionally, the available data were insufficient to draw a conclusion on the reproductive toxicity of fenugreek seed powder in animals, as no reliable NOAEL for these effects was demonstrated.

Additional results of literature searches were provided in the addenda of October 2007 and February 2009, and discussed in May 2009. The experts considered that these did not provide more information to conclude on the minimum active dose with regard to potential reproductive effects.

2.7. Neurotoxicity

In the DAR, no data on neurotoxicity were available. They were not considered necessary during the peer-review.

2.8. Further studies

In the DAR, no further studies were available.

2.9. Medical data

Published reviews indicate that fenugreek seeds are used for culinary and medicinal purposes (see France, 2009). Among the medicinal uses, fenugreek seeds have been shown to have hypocholesterolemic activity thought to be due to saponin-rich subfractions.

In addition, the seeds and some of their fractions display a hypoglycaemic effect in diabetic patients. Isolated fibres, saponins and other proteins as well as 4-hydroxyisoleucine, a novel amino acid extracted from the seeds, could be implicated in the hypoglycaemic effect.

In a study with diabetic patients by Sharma (see Volume 3 B.6.8.1 in France, 2004), fenugreek seeds were administered at a daily dose of 25 g during 24 weeks. The investigations were limited but didn't indicate any clinical, hepatic or renal toxicity, and no haematological abnormalities (only a few minor gastrointestinal symptoms).

EFSA notes: In published articles related to the use in humans and quoted in the addenda of October 2006, October 2007 and February 2009, no clinically significant harmful adverse effects have been reported, but several potential side effects during the use of fenugreek as a medication were outlined:

- potential for allergy in sensitized patients or in patients with asthma (due to possible cross-reactivity with chickpeas);
- may increase the risk of bleeding by raising prothrombin time (because fenugreek preparations can contain coumarin derivatives);
- and potential uterine stimulating properties observed in an early animal study (see above in 2.6).

2.10. Acceptable daily intake (ADI), acceptable operator exposure level (AOEL) and acute reference dose (ARfD)

Considering the uncertainties related to the potential hormonal and reproductive effects, and the limitations of the short-term studies, the experts agreed that no reference values could be derived based on the available data. Furthermore the background level of exposure to fenugreek seed powder from culinary use in the general population in Europe is not known. Therefore the risk assessment has to be considered inconclusive.

2.11. Dermal absorption

In the DAR, a dermal absorption value of 10 % was proposed, based on the composition of fenugreek seed powder. However, the experts agreed that a dermal absorption value of 100 % should be used in the absence of valid experimental data.

2.12. Exposure to operators, workers and bystanders

The representative plant protection product 'Stimulia' contains 100 % fenugreek seed powder. The supported use is one to four applications of 1.5 kg a.s./ha diluted in 300L of water, by foliar spray to grapevine (field and greenhouse).

EFSA notes: The risk assessment for the operators, workers and bystanders could not be finalised for the field use since reference values could not be determined. No exposure estimates for the greenhouse use were provided in the DAR.

Operator

The exposure estimates for the field use provided in the DAR were discussed by the experts during the PRAPeR 4 meeting. Revised calculations with UK POEM were provided in the addendum of October 2006, but they were not agreed during the PRAPeR 9 meeting since no AOEL had been derived for fenugreek seed powder. Therefore it should be noted that the following results are not expressed as percent of AOEL but are the revised estimates in mg/kg bw/day (systemic exposure), and that the risk assessment cannot be concluded.

Results of systemic exposure estimates, presented in mg/kg bw/day

Crop	Method of application	Model	No PPE	PPE*
Grapevine (field)	tractor mounted sprayer 300 L/ha, 15 ha/d, 6 h/d	UK POEM	15.5 mg/kg bw/day	7.4 mg/kg bw/day
	hand-held sprayer (15L tank) 300 L/ha, 1 ha/d, 6 h/d	UK POEM	12.8 mg/kg bw/day	4.2 mg/kg bw/day

* PPE (personal protective equipment): gloves during mixing/loading and application

Bystander

The bystander exposure estimates were provided in the addendum of October 2006. At 10 m from the field, assuming an exposed body surface of 1 m² and a body weight of 60 kg, the resulting systemic exposure was 0.015 mg/kg bw.

Worker

Re-entry exposure estimates for the worker performed with EUROPOEM II were presented in the addendum of October 2006 (France, 2009). Using a transfer factor of 5000 cm²/person/h, a working time of 8 h/day and a body weight of 60 kg, the resulting systemic exposure of the worker was 12 mg/kg bw/day (considering four applications and assuming that all the dry foliar residues accumulate) without the use of gloves.

3. Residues

Fenugreek seed powder was discussed by the experts in residues in the meetings PRAPeR 5 in September 2006 and PRAPeR 10 in November 2006. An update on points that had to be addressed in an addendum as follow up to the meetings PRAPeR 5 and 10 was given by the rapporteur Member State in the meeting of experts PRAPeR 70 in May 2009.

3.1. Nature and magnitude of residues in plant

3.1.1. Primary crops

As the notified active substance is the powdered seed of the fenugreek plant it consists of a complex mixture of natural substances. An investigation of how fenugreek seed powder is metabolised when applied to plants is therefore very difficult.

In a 2003 study conducted by the University of Montpellier the potential transfer of components of fenugreek seed upon application to vine plants was investigated by tracing steroidal saponin and trigonelline markers (see Addendum 2 of December 2006 in France, 2009). Upon application of fenugreek seed powder to young vine leaves by spraying or by infiltration, treated and untreated leaves, stems and roots were sampled during a 96 hour period after treatment and subjected to analyses. Both the chosen marker compounds trigonelline and 4-hydroxyisoleucine were detected only in the treated leaves of the vine plants. Therefore, it was concluded that with conditions as used in this experiment, components in fenugreek seed powder were not systemic when applied to vine leaves.

Following the meeting PRAPER 5 the applicant provided an explanation of the mode of action as a receptor mediated stimulation of the resistance of the plant. It was stated that fenugreek seed powder is acting on the plant, and not chemically on the fungus. Thus the experts concluded that the active substance and compound of concern is fenugreek seed powder.

The study of fenugreek composition shows that it contains only natural substances present in most plants. It is reasonable to consider that these natural substances are degraded in/on treated grapevine plants in accordance with the known metabolic pathways for plant matter and this degradation leads to simple components also present in the natural environment.

Therefore it was concluded that the pertinent residue on treated crops was fenugreek seed powder and this should be the residue definition for risk assessment. Since fenugreek seed powder is a very complex mixture of naturally occurring substances it was the view of the meeting to not propose a residue definition for monitoring and MRL's.

No residue trial data are available. The rapporteur Member State proposed that no significant residues of fenugreek seed powder would be present in vine grapes because treatment is stopped at the flowering stage, i.e. approximately 12 weeks before harvest. According to current guidance on data requirements for active substances made from plants or plant extracts, residue data are only required in cases where relevant residues occur on treated plants, and consumer exposure due to the use as a plant protection product is significant. This was considered not to be the case for the use of fenugreek seed powder.

No data on processing were submitted or required.

3.1.2. Succeeding and rotational crops

Data to address this area of assessment is not relevant as usually there is no crop rotation in the vineyard. However, fenugreek seed powder is the powdered seed of the fenugreek plant and thus consists of a complex mixture of natural substances. It is reasonable to consider that the components are degraded in soil in accordance with the known metabolic pathways for plant matter and this degradation leads to simple components ubiquitously present in the environment.

3.2. Nature and magnitude of residues in livestock

Data to address this area of assessment are not relevant as the treated crops are not indented to be fed to animals.

3.3. Consumer risk assessment

To assess consumer risk from the use of fenugreek seed powder as a plant protection product two possible approaches were considered in the peer review:

1) Comparison of estimated consumer exposure to toxicological reference values (conventional approach).

2) Comparison of the extent of exposure due to the use as a plant protection product with the exposure due to the consumption of the plant part itself (suggested by current guidance).

Exposure assessment

In the absence of residue trials data, the rapporteur Member State estimated residue levels that could occur on grapes and vine leaves on the basis of the application rate, crop density and surface, assuming no wash-off and degradation of fenugreek seed powder occurred up to harvest and under processing. On the basis of these worst case assumptions, and with consumption data for table grapes, wine and vine leaves (Greece) the chronic and acute consumer intake of fenugreek seed powder was estimated to range between approximately 10 - 600 mg/person/day.

The actual consumer exposure due to the use of fenugreek seed powder as a plant protection product is likely to be much lower; however more precise estimates have not been possible on the basis of the available information.

Risk assessment

1) Considering the uncertainties related to the potential hormonal and reproductive effects of fenugreek seeds, and the limitations of the short-term studies, the meeting of experts in toxicology agreed that no reference value could be derived based on the available data (refer to section 2.10).

In the available toxicological studies adverse effects are reported at higher levels than the estimated intake of fenugreek seed powder. However, without agreed toxicological reference values a comparison with the estimated consumer exposure levels is currently not possible, and a definite conclusion can not be drawn in this way.

2) Following the approach outlined in current guidance, the estimated exposure to fenugreek seed powder when used as plant protection product was compared to the consumption of the plant part itself.

It appears that in the European countries fenugreek seeds are mainly consumed through spice mixtures. However, spices are used in very low amounts and rarely reported in food consumption surveys. Exposure due to consumption of fenugreek seeds in the diet could not be determined.

It was noted that fenugreek is also used as a dietary supplement or in medicinal products; however both these products are usually sold to the consumer with a warning label aimed at pregnant or nursing women and at persons taking medication, undergoing surgery or having a medical condition.

The recommended dosage as a dietary supplement or as a medicinal product varies between 600 mg and several grams per person per day according to the desired effects.

On the basis of the use of fenugreek seeds in dietary supplements or in medicinal products the rapporteur Member State considered the exposure to fenugreek seed powder from use as a plant protection product would be of no risk for the consumer, since the estimated intakes of fenugreek seed powder (between 10 - 600 mg/person/day) when used in plant protection were based on worst case assumptions.

The assessment approach presented by the rapporteur Member State seemed appropriate to the majority of experts in the meeting PRAPeR 5, however a minority of experts was of a different

opinion, referring to the potential hormonal and reproductive effects of fenugreek seeds, and the uncertainty in terms of an acceptable dose for consumers as highlighted in the section on toxicology. The consumer risk assessment was therefore inconclusive.

3.4. Proposed MRLs

As fenugreek seed powder is a very complex mixture of naturally occurring substances, the meeting of experts PRAPeR 10 did not propose a residue definition for monitoring and an MRL. If the comparative consumer risk assessment as presented in this conclusion were acceptable to risk managers, fenugreek seed powder could be considered a candidate for Annex IV of Council Regulation (EC) No 369/2005.[6]

4. Environmental fate and behaviour

The fate and behaviour of fenugreek seed powder was discussed in the meeting of experts PRAPeR 2 (September 2006) on basis of the DAR. Updated PECs presented by the RMS in addendum B8 (October 2009; France 2009) are considered as peer reviewed in this conclusion.

No experimental data were submitted on fate and behaviour.

4.1. Fate and behaviour in soil

The applicant provided a case study with the following elements:

'The fenugreek seed contains natural substances present in most plants. It is reasonable to consider that the different natural substances are degraded in soil in accordance with the known metabolic pathways in living matter and this degradation leads to simple components also present in the natural environment.

'It is also underlined that there is no report about pollution by fenugreek seeds near fenugreek crops: during its annual cycle, fenugreek seeds fall from the arborescence at the end of maturation and are scattered on the surrounding ground. The natural substances present in the seeds integrate the natural vegetal cycle to be decomposed into simple components.

'Generally, the harvest of fenugreek seeds reaches 4000 kg per hectare. It is reasonable to consider that, in this case, the level of the seeds debris on the soil is higher than the amount of the plant protection product which falls on the soil after treatment of grapevine, in view of the utilized dose (1.5 kg per hectare).'

As no DT_{50} was available, only initial PEC soil were derived for the risk assessment of its use in grapevine. The PEC_{soil} were in the range of 2 to 8 mg fenugreek seed powder/kg soil.

4.1.1. Mobility in soil of the active substance and their metabolites, degradation or reaction products

Considering the nature of the substance no movement of fenugreek seed powder in soil is expected.

4.2. Fate and behaviour in water

4.2.1. Surface water and sediment

In addition to the previous elements, the applicant provided the following argumentation:

6 Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC.

'The fenugreek is a plant of natural habitat which can be present near an aquatic environment. It is very probable that the seeds can be transported on water surface. There is no report about obvious phenomena of water contamination by the fenugreek seeds near fenugreek fields.'

As no DT_{50} is available, initial PEC_{SW} were calculated assuming spray drift at 3 m of the edge of a static 30 cm depth water body following the GAPs for the grapevine representative uses proposed. Calculated PEC_{SW} ranged from 40.1 to 160.4 µg / L.

4.2.2. Potential for ground water contamination of the active substance, their metabolites, degradation or reaction products

Due to the nature of fenugreek seed powder it is not expected to have the potential to contaminate ground water by leaching.

4.3. Fate and behaviour in air

Due to the nature of fenugreek seed powder it is not expected to have the potential to contaminate the air compartment.

5. Ecotoxicology

Fenugreek seed powder was discussed at the PRAPeR 3 experts' meeting for ecotoxicology in September 2006 on the basis of the DAR and the addendum from August 2006. This addendum was subsequently revised in October 2009.

Fenugreek seed powder is the seed powder of fenugreek (*Trigonella foenum graecum* L.). The supported use evaluated was as plant activator on grapevine (field and greenhouse). The representative product was 'Stimulia'; the maximum application rate was 1.5 kg a.s./ha (4 maximum applications, 10 days of interval).

No long-term studies on birds, aquatic organisms or earthworms were provided for fenugreek seed powder, and no studies were provided on non-target arthropods, other soil macro-organisms, flora and fauna, and biological methods of sewage treatment. The Member State experts considered that further studies were not required since specific inhibition or deleterious effects were not expected, no residues in plant were generated, and residues in soil could be considered not higher than those expected by crops of fenugreek itself.

No risk assessment was provided for greenhouse use but it could be considered negligible.

5.1. Risk to terrestrial vertebrates

On the basis of the acute study on Japanese quail (*Coturnix coturnix japonica*), fenugreek seed powder was not toxic to birds: no mortality was observed at the highest tested dose (LD_{50} >2000 mg a.s./kg bw). The TER_a calculated for insectivorous birds was above the Annex VI trigger, indicating a low risk.

Fenugreek seed powder was not toxic to mammals (acute oral LD_{50} in rat >5000 mg a.s./kg bw, dietary 90-days NOAEL 2500 mg a.s./kg bw). The TERs calculated for small herbivorous mammals were above the Annex VI triggers, indicating a low acute and chronic risk.

5.2. Risk to aquatic organisms

Acute studies were provided on fish (*Oncorhynchus mykiss*), invertebrates (*Daphnia magna*) and algae (*Scenedesmus subspicatus*), indicating a low toxicity of fenugreek seed powder to aquatic

organisms. TERs calculated on the basis of the worst case PEC_{sw} value (see section 4.2) were well above the Annex VI triggers, indicating a low risk.

5.3. Risk to bees

Acute and contact toxicity studies were provided indicating a low toxicity to bees (*Apis mellifera*). The HQ values were below the Annex VI trigger of 50 and therefore risk to bees could be considered low.

5.4. Risk to other non-target arthropod species

No data were provided on the risk to other non-target arthropod species. Further data were considered not necessary by the Member State experts. EFSA notes that the applicant did mention a glass plate study with *Typhlodromus pyri* in the evaluation table. This study was not submitted by the applicant and it has not been assessed by the rapporteur Member State.

5.5. Risk to earthworms

On the basis of an acute toxicity study on *Eisenia foetida*, fenugreek seed powder was not toxic to earthworms (14 days LC_{50} > 5000 mg/kg dry soil). TERs calculated with the worst case PEC_{soil} value (see section 4.1) were well above the Annex VI trigger, indicating a low risk.

5.6. Risk to other soil non-target macro-organisms

No data were provided on the risk to other soil non-target macro-organisms. Further data were considered not necessary by the Member States experts.

5.7. Risk to soil non-target micro-organisms

No effects of greater than 25 % on soil respiration and nitrification were observed in a test with fenugreek seed powder up to concentrations equivalent to four times the application rate proposed, indicating a low risk to soil non-target micro-organisms.

5.8. Risk to other non-target-organisms (flora and fauna)

No data were provided on the risks to flora and fauna. Further data were considered not necessary by the Member State experts.

5.9. Risk to biological methods of sewage treatment

No data were provided on the risk to biological methods of sewage treatment. Further data were considered not necessary by the Member State experts.

6. Residue definitions

6.1. Soil

Definition for risk assessment: fenugreek seed powder

Definition for monitoring: none proposed

6.2. Water

6.2.1. Ground water

Definition for exposure assessment: none proposed

Definition for monitoring: none proposed

6.2.2. Surface water

Definition for risk assessment:

in surface water: fenugreek seed powder

in sediment: fenugreek seed powder

Definition for monitoring: none proposed

6.3. Air

Definition for risk assessment: none proposed

Definition for monitoring: none proposed

6.4. Food of plant origin

Definition for risk assessment: fenugreek seed powder

Definition for monitoring: none proposed, not considered feasible

6.5. Food of animal origin

Definition for risk assessment: not applicable

Definition for monitoring: not applicable

6.6. Overview of the risk assessment of compounds listed in residue definitions for the environmental compartments

6.6.1. Soil

Compound (name and/or code)	Persistence	Ecotoxicology
Fenugreek seed powder	No data available, expected to decompose naturally in soil together with other vegetal crop residues.	Risk to earthworms and non-target micro-organisms was assessed as low.

6.6.2. Ground water

Compound (name and/or code)	Mobility in soil	>0.1 µg/L 1m depth for the representative uses (at least one FOCUS scenario or relevant lysimeter)	Pesticidal activity	Toxicological relevance	Ecotoxicological activity
None					

6.6.3. Surface water and sediment

Compound (name and/or code)	Ecotoxicology
Fenugreek seed powder	No toxic effects on aquatic organisms (acute $E(L)C_{50}$ > 100 mg a.s./L). The risk to aquatic organisms was assessed as low, as was the potential for bioaccumulation.

6.6.4. Air

Compound (name and/or code)	Toxicology
None	

LIST OF STUDIES TO BE GENERATED, STILL ONGOING OR AVAILABLE BUT NOT PEER REVIEWED

- The concentration ranges for marker compounds should be given (relevant for all uses evaluated, data requirement identified by meeting of experts November 2006, proposed submission date unknown; see section 1).
- Specification for mycotoxins supported by batch data (relevant for all uses evaluated, data requirement identified by meeting of experts November 2006, proposed submission date unknown; see section 1).
- Shelf life study to include quantification of the marker compounds and analysis of mycotoxins (relevant for all uses evaluated, data requirement identified by meeting of experts November 2006, data submitted and evaluated in the 2009 addendum Volume 3 B.2 however this data has not been peer reviewed; see section 1).
- Sprayability study (relevant for all uses evaluated, data requirement identified by meeting of experts November 2006, proposed submission date unknown; see section 1).
- Clarification of the formulation type (relevant for all uses evaluated, data requirement identified by meeting of experts November 2006, proposed submission date unknown; see section 1).
- Infra-red spectrum of fenugreek powder (relevant for all uses evaluated, data requirement identified by meeting of experts November 2006, proposed submission date unknown; see section 1)
- Structures for the specific marker compounds (relevant for all uses evaluated, data gap identified by meeting of experts November 2006, proposed submission date unknown; see section 1).
- Clarification of the hormonal activity and reproductive toxicity of fenugreek seed powder is needed, including demonstration of a reliable NOAEL for these effects (relevant for all representative uses evaluated; see section 2.6)

CONCLUSIONS AND RECOMMENDATIONS

OVERALL CONCLUSIONS

This conclusion was reached on the basis of the evaluation of the representative uses as a plant activator on grapevines. Full details of the GAP can be found in the list of end points.

The representative formulated product for the evaluation was ‘Stimulia’, the applicant proposed that the formulation is a soluble powder (SP). The formulation was questioned during the peer review process and the formulation type remains unclear.

Methods of analysis for monitoring are not required due to the nature of this product. The majority of the physical-chemical properties data were considered to be not relevant for this product. It was agreed that this product should be specified as fenugreek seed powder 100%. Marker components were selected for quality control purposes but concentration ranges for these components were not agreed. Also a specification limit for mycotoxins has not been agreed.

Sufficient analytical methods, as well as methods and data relating to physical, chemical and technical properties are available to ensure that some quality control measurements of the plant protection product are possible. There are some storage stability data outstanding although it is noted that these are available in the 2009 addendum to Volume 3 B.2. but not peer reviewed. A sprayability

study for the formulation is needed as the product failed the wet sieve test. An infra-red spectrum is also a data requirement.

With regard to the mammalian toxicity assessment, most of the results/information was from published literature. Tests performed in 2006 demonstrated the absence of skin or eye irritating properties and no mutagenic effect was observed in an Ames test. The experts agreed that the powder had a low acute oral toxicity in mice and rats. Published short-term rat studies derived a NOAEL of 2500 mg/kg bw/day but no raw data were available and the females had not been investigated. The available information did not address the concern regarding potential reproductive toxicity and hormonal activity. Therefore the experts agreed that insufficient data were available to demonstrate a reliable NOAEL for these effects and no reference values were derived. Consequently, the risk assessment for the operators, bystanders and workers can not be concluded.

Limited experimental data are available on the fate of fenugreek seed powder when applied to grapevine. Fenugreek seed powder is not considered to act systemically in plants. It contains natural substances and it seems reasonable to assume that these substances are degraded according to the known metabolic pathways for plant matter. Therefore, it was concluded that the pertinent residue on treated crops to be considered in a consumer risk assessment is fenugreek seed powder. The notified use in grapevine did not require the assessment of residues in following crops or in livestock. It was agreed not to propose a residue definition for monitoring and MRL's for residues of fenugreek seed powder in grapevine.

No data on the amounts of fenugreek seed powder present on the crop at the time of harvest were submitted. Hence, estimates were made on the basis of the application rate, crop density and surface area assuming no wash-off or degradation occurred until harvest. On this basis, approximate values for consumer intake of fenugreek seed powder from treated grapes and leaves were estimated.

Since no toxicological reference values could be established for fenugreek seed powder a comparison of estimated and acceptable daily intake for the consumer has not been not possible. There are also no data available to compare the estimated consumer intake following a pesticidal use of fenugreek seed powder to the amounts of fenugreek seed spice usually consumed in the human diet. The only parameters available for comparison are doses administered in medical practice, or as a dietary supplement. However these applications are always intended to serve a particular purpose and such products usually carry a warning label. It was agreed that the consumer intake of fenugreek seed powder in terms of the use on grapevine is overestimated by the theoretical calculations. It could not be agreed by all experts that a comparison to doses of fenugreek seed administered as medical products or dietary supplements was appropriate.

The consumer risk assessment was inconclusive since toxicological reference values could not be established.

No experimental data on the fate and behaviour of fenugreek seed powder were submitted. The risk assessment was performed on basis of estimated worst case initial exposure and on basis of the following case elements presented by the applicant:

> 'The fenugreek seed contains natural substances present in most plants. It is reasonable to consider that the natural substances are degraded in soil in accordance with the known metabolic ways in living matter and this degradation leads to simple components also present in the natural environment.
>
> 'It is also underlined that there is no report about pollution by fenugreek seeds near fenugreek crops: during its annual cycle, fenugreek seeds fall from the arborescence at the end of maturation and are scattered on the surrounding ground. The natural substances present in the seeds integrate the natural vegetal cycle to be decomposed into simple components.
>
> 'Generally, the harvest of fenugreek seeds reaches 4000 kg per hectare. It is reasonable to consider that, in this case, the level of the seeds debris on the soil is higher than the amount

of the plant protection product which falls on the soil after treatment of grapevine, in view of the utilized dose (1.5 kg per hectare).'

The risk to non-target organisms was expected to be low for the representative use evaluated.

PARTICULAR CONDITIONS PROPOSED TO BE TAKEN INTO ACCOUNT TO MANAGE THE RISK(S) IDENTIFIED

Since the risk assessment for operators, workers and bystanders could not be finalised the need for particular conditions to be taken into account to manage the risk could not be identified.

ISSUES THAT COULD NOT BE FINALIZED

- A specification for mycotoxins.
- Evaluation of the hormonal activity of fenugreek seed powder, including the identification of the minimum active dose on the uterine muscle and characterization of the risk to pregnant women (see section 2.6).
- The operator, worker and bystander risk assessment.
- Although the consumer risk assessment was finalized it was inconclusive.

CRITICAL AREAS OF CONCERN

No toxicological reference values could be allocated (no ADI, no AOEL, and no ARfD); therefore the risk assessment (operator, worker and bystander) is inconclusive (see section 2.10).

REFERENCES

France, 2004. Draft Assessment Report (DAR) on the active substance FEN 560 (Fenugreek seed powder) prepared by the rapporteur Member State France in the framework of Directive 91/414/EEC, December 2004.

France, 2009. Final addendum to Draft Assessment Report on fenugreek seed powder (FEN 560), compiled by EFSA, November 2009.

EFSA (European Food Safety Authority), 2009. Peer Review Report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance fenugreek seed powder (FEN 560). Available from www.efsa.europa.eu.

APPENDICES

APPENDIX A – LIST OF ENDPOINTS FOR THE ACTIVE SUBSTANCE AND THE REPRESENTATIVE FORMULATION

Identity, Physical and Chemical Properties, Details of Uses, Further Information

Active substance (ISO Common Name) ‡	**Fenugreek seed powder (formerly FEN 560)**
Function (*e.g.* fungicide)	Plant activator
Rapporteur Member State	France
Co-rapporteur Member State	-

Identity (Annex IIA, point 1)

Chemical name (IUPAC) ‡	None. The active substance is prepared from the seed powder of fenugreek (*Trigonella foenum graecum* L.), a leguminous plant. This product is a complex mixture of chemical substances
Chemical name (CA) ‡	None
CIPAC No ‡	None
CAS No ‡	None
EC No (EINECS or ELINCS) ‡	None
FAO Specification (including year of publication) ‡	None
Minimum purity of the active substance as manufactured ‡	100 % fenugreek seed powder without any additive and no extraction; the seed being of human food grade quality. 3 representative markers: - trigonelline - 4-hydroxyleucine - total proteins
Identity of relevant impurities (of toxicological, ecotoxicological and/or environmental concern) in the active substance as manufactured	Open for mycotoxins
Molecular formula ‡	Not relevant

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Molecular mass ‡	Not relevant
Structural formula ‡	Not relevant

Physical and chemical properties (Annex IIA, point 2)

Melting point (state purity) ‡	Not relevant
Boiling point (state purity) ‡	Not relevant
Temperature of decomposition (state purity)	Not relevant
Appearance (state purity) ‡	Yellow, powder
Vapour pressure (state temperature, state purity) ‡	Not relevant
Henry's law constant ‡	Not relevant
Solubility in water (state temperature, state purity and pH) ‡	Not relevant
Solubility in organic solvents ‡ (state temperature, state purity)	Not relevant
Surface tension ‡ (state concentration and temperature, state purity)	Not relevant
Partition co-efficient ‡ (state temperature, pH and purity)	Not relevant
Dissociation constant (state purity) ‡	Not measured but in solution in water at 20 °C pH of solution is 5.8
UV/VIS absorption (max.) incl. ε ‡ (state purity, pH)	Two maximal O.D. at 369 nm and 287 nm Not really specific because it is a vegetable extract
Flammability ‡ (state purity)	Not highly flammable
Explosive properties ‡ (state purity)	Not-explosive
Oxidising properties ‡ (state purity)	not oxidising

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Summary of representative uses evaluated (*Fenugreek seed powder*)*

Crop and/or situation	Product Name		F G or I	Pests or Group of pests controlled	Formulation		Application				Application rate per treatment			PHI (days)	Remarks
(a)			(b)	(c)	Type (d-f)	Conc. of a.s. (i)	Method Kind (f-h)	Growth stage & season (j)	Number min max (k)	Interval between apps. (min)	kg a.s./hL min max	water (L/ha) min max	kg a.s./ha min max (l)	(m)	
Grapevine	France	STIMULIA	F G	Foliar fungi i.e. Powdery mildew *(Uncinula necator)*	Water Soluble Powder (1)	100 %	Foliar spray	Until flowering	1 - 4	10 days	500 g/hL	300 L/ha	1.5 kg / ha	90	See note 1.

1. It is recommended to spray leaves of grapevine plants with STIMULIA for 3 or 4 applications against powdery mildew at 10 day intervals.

* For uses where the column "Remarks" is marked in grey further consideration is necessary. Uses should be crossed out when the applicant no longer supports this use(s).

(a) For crops, the EU and Codex classifications (both) should be taken into account; where relevant, the use situation should be described (e.g. fumigation of a structure)

(b) Outdoor or field use (F), greenhouse application (G) or indoor application (I)

(c) *e.g.* biting and suckling insects, soil born insects, foliar fungi, weeds

(d) *e.g.* wettable powder (WP), emulsifiable concentrate (EC), granule (GR)

(e) GCPF Codes - GIFAP Technical Monograph No 2, 1989

(f) All abbreviations used must be explained

(g) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench

(h) Kind, *e.g.* overall, broadcast, aerial spraying, row, individual plant, between the plant- type of equipment used must be indicated

(i) g/kg or g/L. Normally the rate should be given for the active substance (according to ISO) and not for the variant in order to compare the rate for same active substances used in different variants (e.g. fluoroxypyr). **In certain cases, where only one variant is synthesised, it is more appropriate to give the rate for the variant (e.g. benthiavalicarb-isopropyl).**

(j) Growth stage at last treatment (BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application

(k) Indicate the minimum and maximum number of application possible under practical conditions of use

(l) The values should be given in g or kg whatever gives the more manageable number (e.g. 200 kg/ha instead of 200 000 g/ha or 12.5 g/ha instead of 0.0125 kg/ha

(m) PHI - minimum pre-harvest interval

Methods of Analysis

Analytical methods for the active substance (Annex IIA, point 4.1)

Technical as (analytical technique)	Note: fenugreek seed powder is a vegetable extract and the active ingredient was not really identified. However three markers are used to characterize fenugreek seed powder: Trigonelline HPLC method with UV detection (265 nm). The principle of the method is a reverse phase chromatography (water (pH 3)/Methanol; 50/50; v/v) with a LiCrirosorb SI 60-5 column (250 x 4.6 mm, 5 µm). A standard of trigonelline (batch number CGCR1-03) was used as external standard. Total proteins The analytical method was based on the Bradford protein assay. The reference item was albumine. The Bradford protein assay is a dye-binding assay based on the differential colour change of a dye in response to a concentration of a given protein. 4-Hydroxyisoleucine An analytical method using HPLC with visible detector (463 nm) was developed to quantify 4-hydroxyisoleucine in fenugreek seed powder. The principle of the method is a reverse phase chromatography (ammonium acetate 0.02 M in water/acetonitrile; 70/30; v/v) with a LC-18 column (250 x 4.6 mm, 5 µm). A standard of 4 – hydroxyisoleucine was used as external standard.
Impurities in technical as (analytical technique)	ELISA methods for mycotoxins Ochratoxin A ELISA (RIDACREEN) Extraction method AOACn° 2000.03 Acetonitrile/water + purification by immuno affinity column Aflatoxin B1

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

	ELISA (RIDACREEN) Extraction method Methanol/Water + purification by immuno affinity column <u>Total aflatoxins</u> ELISA (RIDACREEN) Extraction method Methanol/Water + purification by immuno affinity column Open methods not peer reviewed
Plant protection product (analytical technique)	No method developed. The plant protection product is totally similar to the active substance

Analytical methods for residues (Annex IIA, point 4.2)

Residue definitions for monitoring purposes

Food of plant origin		No data submitted. Not required
Food of animal origin		No data submitted. Not required
Soil		No data submitted. Not required
Water	surface	No data submitted. Not required
	drinking/ground	No data submitted. Not required
Air		No data submitted. Not required

Monitoring/Enforcement methods

Food/feed of plant origin (analytical technique and LOQ for methods for monitoring purposes)	No data submitted. Not required
Food/feed of animal origin (analytical technique and LOQ for methods for monitoring purposes)	No data submitted. Not required
Soil (analytical technique and LOQ)	No data submitted. Not required

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Water (analytical technique and LOQ)	No data submitted. Not required
Air (analytical technique and LOQ)	No data submitted. Not required
Body fluids and tissues (analytical technique and LOQ)	No data submitted. Not required

Classification and proposed labelling with regard to physical and chemical data (Annex IIA, point 10)

	rapporteur Member State/peer review proposal
Active substance	None

Impact on Human and Animal Health

Absorption, distribution, excretion and metabolism in mammals (Annex IIA, point 5.1)

Rate and extent of absorption:	No data – not required
Distribution:	No data – not required
Potential for accumulation:	No data – not required
Rate and extent of excretion:	No data – not required
Metabolism in animals	No data – not required
Toxicologically significant compounds	Fenugreek seed powder

Acute toxicity (Annex IIA, point 5.2)

Rat LD_{50} oral	> 5000 mg/kg (Defatted fenugreek seed powder)
Rat LD_{50} dermal	No data – not required
Rat LC_{50} inhalation	No data – not required
Skin irritation	Non irritant

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Eye irritation	Non irritant
Skin sensitization (test method used and result)	Non sensitiser (LLNA).

Short-term toxicity (Annex IIA, point 5.3)

Target / critical effect	None
Relevant oral NOAEL	2500 mg/kg (90-day oral rat) – study of poor quality
Relevant dermal NOAEL	No data – not required
Relevant inhalation NOAEL	No data – not required

Genotoxicity (Annex IIA, point 5.4)	Negative (Ames test)

Long-term toxicity and carcinogenicity (Annex IIA, point 5.5)

Target/critical effect	No data
Relevant NOAEL	No data
Carcinogenicity	No data – not required

Reproductive toxicity (Annex IIA, point 5.6)

Reproduction target / critical effect	Insufficient data. Potential for reproductive toxicity in humans cannot be fully excluded based on the available data and indications from the therapeutic use of fenugreek.
Relevant reproductive NOAELs	Not derivable from the available information.

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Developmental target / critical effect	No data, not required
Relevant developmental NOAEL	None

Neurotoxicity / Delayed neurotoxicity (Annex IIA, point 5.7)

No data, not required

Other toxicological studies (Annex IIA, point 5.8)

Literature data on genotoxicity of 4-hydroxyisoleucine, trigonelline, flavonoids: not mutagenic.

Medical data (Annex IIA, point 5.9)

In medical practice the administered doses of fenugreek seeds were relatively high (>5 g/day for prolonged periods) without any indication of adverse effects unless minor gastrointestinal symptoms such as diarrhoea and excess flatulence which subsided after a few days.

Summary (Annex IIA, point 5.10)	Value	Study	Safety factor
ADI	Not possible to allocate at the moment		
AOEL	Not possible to allocate at the moment		
ARfD (acute reference dose)	Not possible to allocate at the moment		

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Dermal absorption (Annex IIIA, point 7.3)

100 % (no experimental data)

Acceptable exposure scenarios (including method of calculation)

Operator

Use in grapevine plants – UK POEM model

(**systemic exposure in mg/kg bw/day**)

Application	No PPE	PPE (gloves)
By tractor	15.5	7.4
Hand-held	12.8	4.2

Worker

Systemic exposure of 12 mg/kg bw/day

(without PPE)

Bystander

Systemic exposure of 0.015 mg/kg bw.

Classification and proposed labelling (Annex IIA, point 10)

with regard to toxicological data	None

Residues

Metabolism in plants (Annex IIA, point 6.1 and 6.7, Annex IIIA, point 8.1 and 8.6)

Plants groups covered	No data, not necessary. Mixture of natural substances degraded according to known metabolic pathways in living matter
Rotational crops	No data, not necessary
Plant residue definition for monitoring	None, not feasible since complex mixture of natural substances
	Fenugreek seed powder

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Plant residue definition for risk assessment	
Conversion factor (monitoring to risk assessment)	

Metabolism in livestock (Annex IIA, point 6.2 and 6.7, Annex IIIA, point 8.1 and 8.6)

Animals covered	No data, not required
Animal residue definition for monitoring	None (not relevant)
Animal residue definition for risk assessment	None (not relevant)
Conversion factor (monitoring to risk assessment)	None (not relevant)
Metabolism in rat and ruminant similar (yes/no)	No data (not required)
Fat soluble residue (yes/no)	No data. Some components of the active substance are fat soluble.

Residues in succeeding crops (Annex IIA, point 6.6, Annex IIIA, point 8.5)

	No residues were expected.

Stability of residues (Annex IIA, point 6 introduction, Annex IIIA, point 8 introduction)

	Not relevant (No residues were expected)

Residues from livestock feeding studies (Annex IIA, point 6.4, Annex IIIA, point 8.3)

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Intakes by livestock < 0.1 mg/kg diet/day	Ruminant:	Poultry:	Pig:
Muscle	Not relevant	Not relevant	Not relevant
Liver	Not relevant	Not relevant	Not relevant
Kidney	Not relevant	Not relevant	Not relevant
Fat	Not relevant	Not relevant	Not relevant
Milk	Not relevant	Not relevant	Not relevant
Eggs	Not relevant	Not relevant	Not relevant

Summary of critical residues data (Annex IIA, point 6.3, Annex IIIA, point 8.2)

Crop	Northern or Mediterranean Region	Trials results relevant to the critical GAP	Recommendation / comments	MRL mg/kg	STMR mg/kg
Grapevine	-	No data Residue levels on grapes and vine leaves were estimated on the basis of the application rate, crop density and surface, assuming no wash-off and degradation		None proposed	Not applicable

Consumer risk assessment (Annex IIA, point 6.9, Annex IIIA, point 8.8)

ADI	Not possible to allocate
	% ADI open No residue expected at levels higher than

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

TMDI (European diet) (% ADI)	exposure due to the consumption of the plant as medical product or dietary supplements
NEDI (% ADI)	Not relevant
Factors included in NEDI	Not relevant
ARfD	Not possible to allocate
Acute exposure (% ARfD)	% ARfD open No residue expected at levels higher than exposure due to the consumption of the plant as medical product or dietary supplements

Processing factors (Annex IIA, point 6.5, Annex IIIA, point 8.4)

Crop/processed crop	Number of studies	Transfer factor	% Transference *
	Not relevant	Not relevant	Not relevant

Proposed MRLs (Annex IIA, point 6.7, Annex IIIA, point 8.6)

Grapes and vine leaves	None [7]

Fate and Behaviour in the Environment

Route of degradation (aerobic) in soil (Annex IIA, point 7.1.1.1.1)

Mineralization after 100 days	No data submitted, not required.
Non-extractable residues after 100 days	No data submitted, not required.
Relevant metabolites - name and/or code, % of applied (range and maximum)	No data submitted, not required.

7 If the comparative consumer risk assessment as presented under section 3.3 of the EFSA conclusion were acceptable to risk managers, fenugreek seed powder could be considered a candidate for Annex IV of Council Regulation (EC) No 369/2005.

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Route of degradation in soil - Supplemental studies (Annex IIA, point 7.1.1.1.2)

Anaerobic degradation	No data submitted, not required.
Soil photolysis	No data submitted, not required.

Rate of degradation in soil (Annex IIA, point 7.1.1.2, Annex IIIA, point 9.1.1)

Method of calculation	No data submitted, not required.
Laboratory studies (range or median, with n value, with r^2 value)	DT_{50lab} (20°C, aerobic): No data submitted, not required.
	DT_{90lab} (20°C, aerobic): No data submitted, not required.
	DT_{50lab} (10°C, aerobic): No data submitted, not required.
	DT_{50lab} (20°C, anaerobic): No data submitted, not required.
	Degradation in the saturated zone: No data submitted, not required.
Field studies (state location, range or median with n value)	DT_{50f}: No data submitted, not required.
	DT_{90f}: No data submitted, not required.
Soil accumulation and plateau concentration	No data submitted, not required.

Soil adsorption/desorption (Annex IIA, point 7.1.2)

K_f /K_{oc} K_d pH dependence (yes / no) (if yes type of dependence)	No data submitted, not required.

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Mobility in soil (Annex IIA, point 7.1.3, Annex IIIA, point 9.1.2)

Column leaching	No data submitted, not required.
Aged residues leaching	No data submitted, not required.
Lysimeter/ field leaching studies	No data submitted, not required.

PEC (soil) (Annex IIIA, point 9.1.3)

Method of calculation	DT_{50}: not available.
Application rate	Crop: grapevine % plant interception: 0 % Number of applications: 4 Interval (d): 10 Application rate: 4 x 1500 g as/ha (as no DT_{50} is available, only an initial PEC_{max} is calculated)

$PEC_{(s)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	2		8	
Short-term 24h 2d 4d				
Long-term 7d 28d				

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

50d 100d				

Route and rate of degradation in water (Annex IIA, point 7.2.1)

Hydrolysis of active substance and relevant metabolites (DT_{50}) (state pH and temperature)	pH_4____: No data submitted, not required.
	pH_7_____: No data submitted, not required.
	pH_9_____: No data submitted, not required.
Photolytic degradation of active substance and relevant metabolites	No data submitted, not required.
Readily biodegradable (yes/no)	Not applicable
Degradation in water/sediment - DT_{50} water - DT_{90} water - DT_{50} whole system - DT_{90} whole system	No data submitted, not required.
Mineralization	
Non-extractable residues	
Distribution in water / sediment systems (active substance)	
Distribution in water / sediment systems (metabolites)	

PEC (surface water) (Annex IIIA, point 9.2.3)

Method of calculation	DT_{50}: not available.

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Application rate	Crop: grapevine Number of applications: 4 Interval (d): 10 Application rate: 4 x 1500 g as/ha (as no DT_{50} is available, only an initial PEC_{max} is calculated) Depth of water body: 0.3 m
Main routes of entry	8.02 % derive from 1 meter.

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

$PEC_{(sw)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	40.1 µg/L	-	160.4 µg/L	-
Short-term 24h 2d 4d				
Long-term 7d 14d 21d 28d 42d				

PEC (sediment)

Method of calculation	No data submitted, not required.
Application rate	

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

PEC$_{(sed)}$	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial				
Short-term				
Long-term				

PEC (ground water) (Annex IIIA, point 9.2.1)

Method of calculation and type of study (*e.g.* modelling, monitoring, lysimeter)	No data submitted, not required.
Application rate	
PEC$_{(gw)}$	
Maximum concentration	
Average annual concentration	

Fate and behaviour in air (Annex IIA, point 7.2.2, Annex III, point 9.3)

Direct photolysis in air	No data submitted, not required
Quantum yield of direct phototransformation	No data submitted, not required
Photochemical oxidative degradation in air (DT$_{50}$)	
Volatilization	from plant surfaces:
	from soil:

PEC (air)

Method of calculation	No data submitted, not required

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

$PEC_{(a)}$

Maximum concentration	No data submitted, not required

Definition of the Residue (Annex IIA, point 7.3)

Relevant to the environment	Soil: Fenugreek seed powder Water: Fenugreek seed powder

Monitoring data, if available (Annex IIA, point 7.4)

Soil (indicate location and type of study)	New active substance, not available.
Surface water (indicate location and type of study)	New active substance, not available.
Ground water (indicate location and type of study)	New active substance, not available.
Air (indicate location and type of study)	New active substance, not available

Classification and proposed labelling (Annex IIA, point 10)

with regard to fate and behaviour data	None proposed

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Ecotoxicology

Effects on Non-target Species

Effects on terrestrial vertebrates (Annex IIA, point 8.1, Annex IIIA, points 10.1 and 10.3)

Acute toxicity to mammals	LD_{50}: >5000 mg a.s./kg bw (rat)
Sub-chronic toxicity to mammals (90 d)	NOAEL =2500 mg a.s./kg bw (rat)
Acute toxicity to birds	LD_{50} > 2000 mg a.s./kg bw
Dietary toxicity to birds	No data available, not required
Reproductive toxicity to birds	No data available, not required

Toxicity/exposure ratios for terrestrial vertebrates (Annex IIIA, points 10.1 and 10.3)

Application rate (kg as/ha)	Crop	Category (*e.g.* insectivorous bird)	Time-scale	TER	Annex VI Trigger
1.5	grapevines	insectivorous bird	acute	>24.65	10
1.5	grapevines	insectivorous bird	long-term	-	5
1.5	grapevines	small herbivorous mammal	acute	>17.63	10
1.5	grapevines	small herbivorous mammal	long-term	>26.39	5

Toxicity data for aquatic species (most sensitive species of each group) (Annex IIA, point 8.2, Annex IIIA, point 10.2)

Group	Test substance	Time-scale	Endpoint	Toxicity (mg/l)
Laboratory tests				
Oncorhynchus mykiss	fenugreek seed powder	96 h	LC_{50}	283

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Daphnia magna	fenugreek seed powder	48 h	EC_{50}	> 100
Scenedesmus subspicatus	fenugreek seed powder	96 h	EbC_{50} ErC_{50}	> 160
Microcosm or mesocosm tests				
No data available				

Toxicity/exposure ratios for the most sensitive aquatic organisms (Annex IIIA, point 10.2)

Application rate (kg as/ha)	Crop	Organism	Time-scale	Distance (m)	TER	Annex VI Trigger
4 x 1.5	grapevines	*Oncorhyncus mykiss*	acute	3	1764	100
4 x 1.5	grapevines	*Daphnia magna*	acute	3	> 623	100
4 x 1.5	grapevines	*Scenedesmus subspicatus*	acute	3	> 998	10

Bioconcentration

Bioconcentration factor (BCF)	Not required
Annex VI Trigger: for the bioconcentration factor	Not required
Clearance time (CT_{50})	Not required
(CT_{90})	
Level of residues (%) in organisms after the 14 day depuration phase	Not required

Effects on honeybees (Annex IIA, point 8.3.1, Annex IIIA, point 10.4)

Acute oral toxicity	> 50 µg/bee
Acute contact toxicity	> 200 µg/bee

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Hazard quotients for honey bees (Annex IIIA, point 10.4)

Application rate (kg as/ha)	Crop	Route	Hazard quotient	Annex VI Trigger
Laboratory tests				
1.5 1.5	grapevine grapevine	oral contact	<30 <7.5	50 50
Field or semi-field tests				
No data available				

Effects on other arthropod species (Annex IIA, point 8.3.2, Annex IIIA, point 10.5)

Species	Stage	Test Substance	Dose (kg as/ha)	Endpoint	Effect	Annex VI Trigger
Laboratory tests						
No data available						
Field or semi-field tests No data available						

Effects on earthworms (Annex IIA, point 8.4, Annex IIIA, point 10.6)

Acute toxicity	LC_{50} (14 day) = 5000 mg/kg soil NOEC (14 day) = 1000 mg/kg soil
Reproductive toxicity	No data available

Toxicity/exposure ratios for earthworms (Annex IIIA, point 10.6)

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

Application rate (kg as/ha)	Crop	Time-scale	TER	Annex VI Trigger
4 x 1.5	grapevine	acute	1147	10

Effects on soil micro-organisms (Annex IIA, point 8.5, Annex IIIA, point 10.7)

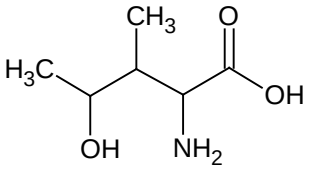
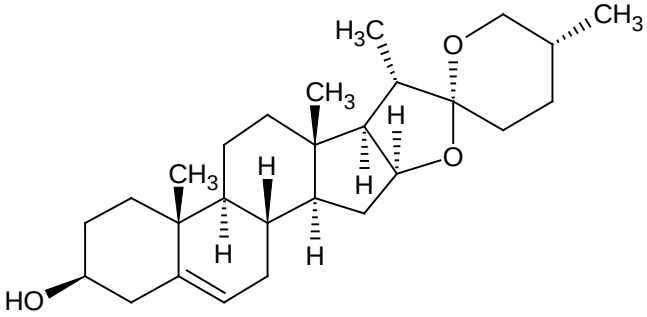
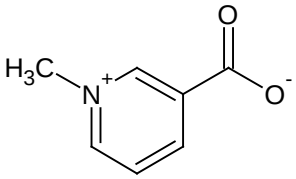
Nitrogen mineralization	No effect after 28 days at 4 times the recommended application rate
Carbon mineralization	No effect after 28 days at 4 times the recommended application rate

Classification and proposed labelling (Annex IIA, point 10)

with regard to ecotoxicological data	None

‡ End point identified by the EU-Commission as relevant for Member States when applying the Uniform Principles

APPENDIX B – USED COMPOUNDS CODES

Code/Trivial name	Chemical name	Structural formula
4-hydroxyisoleucine	2-amino-4-hydroxy-3-methylpentanoic acid	
diosgenin	(3β,25*R*)-spirost-5-en-3-ol	
trigonelline	1-methylpyridinium-3-carboxylate	

ABBREVIATIONS

1/n	slope of Freundlich isotherm
ε	decadic molar extinction coefficient
°C	degree Celsius (centigrade)
µg	microgram
µm	micrometer (micron)
a.s.	active substance
ADE	actual dermal exposure
ADI	acceptable daily intake
AF	assessment factor
AOEL	acceptable operator exposure level
AR	applied radioactivity
ARfD	acute reference dose
AV	avoidance factor
BCF	bioconcentration factor
bw	body weight
CAS	Chemical Abstract Service
CI	confidence interval
CIPAC	Collaborative International Pesticide Analytical Council Limited
CL	confidence limits
d	day
DAA	days after application
DAR	draft assessment report
DAT	days after treatment
DM	dry matter
DT50	period required for 50 percent disappearance (define method of estimation)
DT90	period required for 90 percent disappearance (define method of estimation)
dw	dry weight
EbC50	effective concentration (biomass)
EC50	effective concentration
ECHA	European Chemical Agency
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EMDI	estimated maximum daily intake
ER50	emergence rate/effective rate, median
ErC50	effective concentration (growth rate)
EU	European Union
EUROPOEM	European Predictive Operator Exposure Model
f(twa)	time weighted average factor
FAO	Food and Agriculture Organisation of the United Nations
FIR	Food intake rate
FOCUS	Forum for the Co-ordination of Pesticide Fate Models and their Use
g	gram
GAP	good agricultural practice
GC	gas chromatography
GCPF	Global Crop Protection Federation (formerly known as GIFAP)
GM	geometric mean
GS	growth stage
h	hour(s)
ha	hectare
hL	hectolitre

HPLC	high pressure liquid chromatography or high performance liquid chromatography
HPLC-MS	high pressure liquid chromatography – mass spectrometry
HQ	hazard quotient
IEDI	international estimated daily intake
IESTI	international estimated short-term intake
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemistry
JMPR	Joint Meeting on the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues (Joint Meeting on Pesticide Residues)
Kdoc	organic carbon linear adsorption coefficient
kg	kilogram
KF_{OC}	Freundlich organic carbon adsorption coefficient
L	litre
LC	liquid chromatography
LC50	lethal concentration, median
LC-MS	liquid chromatography-mass spectrometry
LC-MS-MS	liquid chromatography with tandem mass spectrometry
LD50	lethal dose, median; dosis letalis media
LOAEL	lowest observable adverse effect level
LOD	limit of detection
LOQ	limit of quantification (determination)
m	metre
MAF	multiple application factor
mg	milligram
mL	millilitre
mm	millimetre
MRL	maximum residue limit or level
MS	mass spectrometry
MSDS	material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
NESTI	national estimated short-term intake
ng	nanogram
NOAEC	no observed adverse effect concentration
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOEL	no observed effect level
OM	organic matter content
Pa	Pascal
PD	proportion of different food types
PEC	predicted environmental concentration
PEC_{air}	predicted environmental concentration in air
PEC_{GW}	predicted environmental concentration in ground water
PEC_{sed}	predicted environmental concentration in sediment
PEC_{soil}	predicted environmental concentration in soil
PEC_{SW}	predicted environmental concentration in surface water
pH	pH-value
PHI	pre-harvest interval
PIE	potential inhalation exposure
pKa	negative logarithm (to the base 10) of the dissociation constant
P_{OW}	partition coefficient between n-octanol and water

PPE	personal protective equipment
ppm	parts per million (10-6)
ppp	plant protection product
PT	proportion of diet obtained in the treated area
PTT	partial thromboplastin time
QSAR	quantitative structure-activity relationship
r2	coefficient of determination
RUD	residue per unit dose
SC	suspension concentrate
SD	standard deviation
SFO	single first-order
SSD	species sensitivity distribution
STMR	supervised trials median residue
$t_{1/2}$	half-life (define method of estimation)
TER	toxicity exposure ratio
TER_A	toxicity exposure ratio for acute exposure
TER_{LT}	toxicity exposure ratio following chronic exposure
TER_{ST}	toxicity exposure ratio following repeated exposure
TK	technical concentrate
TLV	threshold limit value
TMDI	theoretical maximum daily intake
TRR	total radioactive residue
TWA	time weighted average
UV	ultraviolet
W/S	water/sediment
w/v	weight per volume
w/w	weight per weight
WG	water dispersible granule
WHO	World Health Organisation
wk	week
yr	year