NUTREL/SIC

DOCUMENT M-CP, Section 10

ECOTOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number

[1] It is suggested that applicants adopt a similar approach to showing revisions and version history as outlined in SANCO/10180/2013 Chapter 4 How to revise an Assessment Report

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CP 10.1 Effects on Birds and Other Terrestrial Vertebrates

Effects on Birds

Overview and summary

EU Endpoints: Toxicity of Hydrolysed proteins to birds

Study	Test species	EU agreed endpoints (EFSA Journal 2012; 10(2):2545)
Acute toxicity	Bobwhite quail	LD_{50} (mg/kg bw) No data available
Dietary toxicity (short-term)	Bobwhite quail	LD_{50} (mg/kg bw) No data available
Reproductive toxicity (long-term)	Bobwhite quail	NOEL (mg/kg bw) No data available

NUTREL was not a representative formulation in the EU review of Hydrolysed proteins. An appropriate risk assessment has been provided and are considered adequate.

The risk assessment for effects on birds is carried out according to the 'Guidance of EFSA - Risk assessment for Birds and Mammals' (EFSA 2009) for the worst case use in a crop. The timing of application and the number of applications of NUTREL are the same in all crops. Results in a crop are extrapolated to other crops since the dose rate is the same (300 g a.i./ha vs 1000 g a.i./ha). A summary of the toxicity exposure for birds endpoints is provided in Table 10.1-1.

Table 10.1-1 Toxicity/exposure ratios for birds

Test substance	Crop, use pattern	Indicator species	Toxicity endpoint (mg/kg bw/day)	Short-cut value	DDD (mg/kg bw/day)	TER	TER risk assessment trigger
Hydrolysed proteins	Orchards	Small insectivorous bird (screening step)	LD_{50} = N.A.	N.A	N.A	N.A	N.A
			NOEL = N.A	N.A	N.A	N.A	N.A

Risk Assessment

Toxicity

The product is constituted by natural substances, is completely biodegradable and it's used also as foliar fertiliser. It does not cause negative transformations in the environment, if it is used following the suggested dosages and the suggested conditions.

The product is constituted by natural substances and consequently is completely degradable. Reasonably there are not negative effects on the environment, but if present in copious quantities can pollute ground and surface water: it is necessary to prevent concentrated product from penetrating into ground and surface waters.

NUTREL has not been tested for potential acute toxicity to birds. Birds are typically exposed to dry residues on their food items following the dilution and spraying of the formulated product. During these processes, much of the formulation constituents are likely to be lost by biodegradation. Since oral exposure is the main route of exposure, toxicity data for the active substances are therefore used in preference to data from tests with the formulated material. On this basis, the risk to birds from the proposed uses of -NUTREL will be assessed using data on Hydrolysed proteins.

Avian acute oral and long-term reproduction studies not have been carried out with Hydrolysed proteins. Full details of Hydrolysed proteins avian toxicity studies are provided in **EU DAR**. A summary of the relevant acute and long-term endpoints is provided in Table 10.1-2.

Table 10.1-2: Summary of avian toxicity endpoints for Hydrolysed proteins

Study type	Test substance	Species	Endpoint	Value	Reference
Acute oral toxicity	Hydrolysed proteins	Bobwhite quail	LD_{50}	mg/kg bw N.A	***DAR (2008)****
Long-term toxicity and reproduction	Hydrolysed proteins	Bobwhite quail	NOEL	mg/kg bw N.A	***DAR (2008)****

**Effect on Birds (Annex IIA 8.1; Annex IIIA 10.1)*
Acute oral - Active substance: No studies were submitted
Plant Protection Product: No studies were submitted

Hydrolysed proteins metabolites

Not applicable since Hydrolysed proteins has no relevant metabolites in plants.

In the case hydrolysed proteins of Sicit 2000 SpA, the active ingredients 'Hydrolysed proteins' (means polypeptides, peptides, aminoacids and mixtures thereof), are obtained by the hydrolysis of animal by-products.

Exposure

The potential exposure of birds to Hydrolysed proteins was estimated, in theory, following 2-4 application of NUTREL 297 g/L at 3,050 L product/ha (equivalent to 906 g a.i./ha) or NUTREL 300 g/L at 3,0 L product/ha (equivalent to 900 g a.i./ha) or NUTREL 421 g/L at 2,140 (equivalent to 901 g a.i./ha) in all crops.

The potential exposure of birds to **Hydrolysed proteins** was estimated, in theory, following 2-4 application of NUTREL 378 g/L at 2,4 L product/ha (equivalent to 907 g a.i./ha) in all crops.

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to NUTREL applications is considered unlikely, since at the time of application and for a short period thereafter, most birds will leave the immediate vicinity of spray operations in response to the human disturbance.

Exposure to standard generic indicator species was estimated according to the 'Guidance of EFSA - Risk assessment for Birds and Mammals' (EFSA 2009). The proposed use of NUTREL as a insect attractant in orchards implies that the insects present on these surfaces that are treated with the product may potentially be consumed by insectivores. The appropriate exposure scenario is deemed to be orchards (olive trees, pome fruits, stone fruits, walnut, citrus, fig, kiwi, blueberries). The recommended scenario for grassland at the screnning step is that of a small insectivorous bird with short-cut values of 46.8 and 18.2 for acute and long-term assessments respectively.

According to the EFSA Journal 2012; 10(2):2545, for the DDD (daily dietary dose) to bird: No data available.

In theory, the DDD were calculated by multiplying the application rate (kg/ha) by the short-cut value and the MAF (to account for multiple applications). On top of this, for the long term exposure, the result was multiplied by TWA (factor to account for the time weighted average). This is the theoretical summarized in the following equations:

DDD_{acute} = Application rate (kg a.s./ha) × short-cut value × MAF

DDD_{repro} = Application rate (kg a.s./ha) × short-cut value × MAF × TWA

Where:

- the short-cut value is given by the guidance document
- MAF is the Multiple Application Factor. It is not relevant as the use of NUTREL involves only one application.
- The term TWA is the time-weighted-average factor. This was used to calculate time-weighted average (TWA) residues on leafy crops, which take into account the degradation of the active substances over time. TWA residues were used as an estimate of long-term exposure only, since it is considered that the use of maximum residues provides an unrealistically extreme worst-case estimate of long-term exposure. As worst case, a f_{TWA} of 1 was used as given in the Guidance Document, assuming no degradation of Hydrolysed proteins.

The resulting daily dietary doses are presented in the table 10.1-3.

Table 10.1-3: Daily dietary doses for Hydrolysed proteins at the screening step

Test substance	Crop, use pattern	Indicator species	Assessment type	Short-cut value	DDD (mg/kg bw/day)
Hydrolysed proteins	Orchards	Small insectivorous bird (screening step)	Acute	N.A.	N.A.
			Reproduction	N.A.	N.A.

IIIA 10.1.1 Acute toxicity exposure ratio (TER_A)

The acute risk to birds of Hydrolysed proteins not was assessed by calculating toxicity exposure ratios (TER_A) using the following equation:

$$TER_A = \frac{LD_{50}\ (mg/kg\ bw/day)}{Acute\ DDD\ (mg/kg\ bw/day)}$$

In theory, the resulting TER_A values are given in Table 10.1.1-1.

Table 10.1.1-1: Acute risk (TER_A) to birds (to 2 s.f.) from Hydrolysed proteins

Compound	Scenario	Indicator species	App. rate (g a.s./ha)	LD_{50} (mg a.s./kg bw/day)	Acute DDD (mg a.s./kg bw/day)	TER_A
Hydrolysed proteins	All crops	Small insectivorous bird (screening step)	300-1000	N.A.	N.A.	N.A.

The TER_A value is greater than the Annex VI trigger of 10, indicating low acute risk to birds from Hydrolysed proteins following application of NUTREL at all proposed label rates in all crops.

Review Comments: IIIA 10.1.1/01	
Agreed endpoints: IIIA 10.1.1	

CP 10.1.1.2 Higher tier data on birds

Short and long-term toxicity exposure ratio (TER_{ST})

Short-term toxicity exposure ratio (TER_{ST})

Not required for 6-benzyladenine following the EFSA guidance document. Reference is made to the long term exposure.

Long-term toxicity exposure ratio (TER_{LT})

Long-term toxicity exposure ratios (TER_{LT}) for Hydrolysed proteins following 1 application of NUTREL not were calculated using the following equation:

$$TER_{LT} = \frac{\text{NOEL (mg/kg bw/day)}}{\text{Long - term DDD (mg/kg bw/day)}}$$

In theory, the resulting TER values are given in Table 10.1.2-1.

Table 10.1.1.2-1: Long-term risk (TER_{LT}) to birds from Hydrolysed proteins

Compound	Scenario	Indicator species	App. rate (g a.s./ha)	NOEL (mg a.s./kg bw/day)	Long-term DDD (mg a.s./kg bw/day)	TER_{LT}
Hydrolysed proteins	All crops	Small insectivorous bird (screening step)	300-1000	N.A.	N.A.	N.A.

The TER_{LT} value for Hydrolysed proteins is greater than the Annex VI trigger of 5, indicating NUTREL presents no unacceptable long-term risk to birds when applied in all crops.

Review Comments: IIIA 10.1.1.2/01	
Agreed endpoints: IIIA 10.1.1.2	

CP 10.1.1.2.1 Baits: Concentration of active substance in bait in mg/kg

NUTREL is intended for use as a foliar spray, and therefore this information is not required.

The Hydrolysed protein is used as: a) a foliar spray in mixture with an insecticide, applied on some trees or some branches of trees in orchards and olive groves and b) as a bait for trapping of flies.
The NUTREL formulation product is used in agriculture as an attractant of Diptera in fruit trees (olives, pomme trees, stone trees, citrus, fig and walnut), kiwi and blueberries, in mixture with conventional insecticide/s.
The Hydrolysed protein is a food attractant to insect pests. When applied in mixtures with insecticides, insects are attracted by the protein, and are eventually killed by the insecticide effect. In the case of mass trapping, insects are attracted to a trap from which they cannot escape

CP 10.1.1.2.2 Pellets, granules, prills or treated seed

NUTREL is intended for use as a foliar spray or as bait attractiveness for mass trapping, and therefore this information is not required.

CP 10.1.1.2.3 Amount of active substance in or on each item

NUTREL is intended for use as a foliar spray or as bait attractiveness for mass trapping, and therefore this information is not required.

CP 10.1.1.2.4 Proportion of active substance LD_{50} per 100 items and per gram of items

NUTREL is intended for use as a foliar spray or as bait attractiveness for mass trapping, and therefore this information is not required.

CP 10.1.1.2.5 Size and shape of pellet, granule or prill

NUTREL is intended for use as a foliar spray or as bait attractiveness for mass trapping, and therefore this information is not required.

CP 10.1.1.2.6 Acute toxicity of the formulation

Avian toxicity tests with the formulation were not performed, since it is possible to extrapolate from data obtained with the active substance.

CP 10.1.1.2.7 Supervised cage or field trials

The risk assessment above demonstrated that the proposed use of NUTREL poses no unacceptable risk to birds, and therefore further studies are not considered necessary.

CP 10.1.1.2.8 Acceptance of bait, granules or treated seeds (palatability testing)

The information concerned is not relevant since NUTREL is intended for use as a foliar spray or as bait attractiveness for mass trapping.

CP 10.1.1.2.9 Effects of secondary poisoning

According to the 'Guidance of EFSA - Risk assessment for Birds and Mammals' (EFSA 2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

In the case of Hydrolysed proteins, this is not relevant since Bioconcentration factor (BCF) is not required and, therefore, it is presumably lower at log POW of 3.0 at pH 7. For this reason, no risk of biomagnification in terrestrial food chain is expected following the application of NUTREL in all crops.

CP 10.1.2 Effects on Terrestrial Vertebrates Other Than Birds

CP 10.1.2.1 Acute oral toxiciy to mammals

Overview and summary

EU Endpoints: Effects on Terrestrial Vertebrates Other Than Birds
Ecotoxicological endpoints for mammals

Active substance	Test species[1]	EU agreed endpoints (EFSA Journal 2012; 10(2):2545)	Remark
Acute			
Hydrolysed proteins	Rat	LD_{50} (mg/kg bw)	Data available pf limited validity*
Chronic			
Hydrolysed proteins	Rat	NOAEL (mg/kg bw) (relevant parental and offspring effects)	No data available*

*Hydrolysed proteins *per se* are considered of low toxicological concern and no risks to human health are expected from its use as a plant protection product. However due to the fact that a specification to include the main components in the active substances is still outstanding a final conclusion cannot be drawn whether the technical specification is of toxicological concern leading to a data gap and issue that cannot be finalized.

Summary

NUTREL was not a representative formulation in the EU review of Hydrolysed proteins. An appropriate risk assessment has been provided and is considered adequate.

The risk assessment for effects on mammals is carried out according to the 'Guidance of EFSA - Risk assessment for Birds and Mammals' (EFSA 2009)[1].

[1] EFSA (2009). Guidance of EFSA – Risk assessment for Birds and Mammals. EFSA Journal 2009; 7(12):1438.

Table 10.1.2-1 Toxicity/exposure ratios for mammals

Test substance	Crop, use pattern	Indicator species	Toxicity endpoint (mg/kg bw/day)	Short-cut value	DDD (mg/kg bw/day)	TER	TER risk assessment trigger
Hydrolysed proteins	Orchards	Small herbivorous mammal (screening step)	LD_{50} = N.A.	N.A.	N.A.	N.A.	10
			NOEL = N.A.	N.A	N.A	N.A	5
	Orchard application, crop directed >BBCH 71	Large herbivorous mammal "lagomorph" Non-grass herbs 100% Non-grass herbs (tier 1)	NOEL = N.A.	N.A	N.A	N.A	5
		Small herbivorous mammal "vole Grass + cereals" 100% grass (tier 1)	NOEL = N.A.	N.A	N.A	N.A	5
		Small omnivorous mammal "mouse" Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods (tier 1)	NOEL = N.A.	N.A	N.A	N.A	5

DDD = Defined Daily Dose

In theory, the screening step of the assessment shows an acceptable acute dietary risk of NUTREL to mammals but no acceptable reproductive dietary risk. A tier 1 assessment was therefore triggered for the reproductive risk assessment for applications in orchards at BBCH > 71 resulting in an acceptable risk. The results of these assessments show that the risk to mammals is acceptable.

Risk assessment

Toxicity

The mammalian toxicity endpoints for Hydrolysed proteins that are most appropriate for acute and long-term ecological risk assessment are summarised in the tables 10.3-2 and 10.3-3. Further details can be found in the corresponding *EU review* for Hydrolysed proteins. The EU endpoints for Hydrolysed proteins will be used for the risk assessment.

Table 10.1.2-2: Acute oral toxicity to mammals

Substance	Species	Endpoint	Value (mg/kg bodyweight)	Report[1]
NUTREL	Rat	LD_{50}	No data available*	***DAR (2008)***
Hydrolysed proteins	Rat	LD_{50}	No data available*	***DAR (2008)***

Acute endpoint shown in bold is used in the risk assessment

Table 10.1.2-3: Reproductive toxicity to mammals

Substance	Species	Study Endpoint	Value (mg a.s./kg diet)	DDD (mg a.s./kg bw/d)	Report
Hydrolysed proteins	Rat	2-generation study:NOEC	No data available*	No data available*	***DAR (2008)***

NOAEC = No observed adverse effect concentration;

Hydrolysed proteins

Not applicable since Hydrolysed proteins has no relevant metabolites in plants.

Exposure

The potential exposure of mammals to Hydrolysed proteins was estimated following 2-4 applications of NUTREL (378 g/L) at 2.4 L product/ha (equivalent to 907 g a.s./ha) to reflect the worse case scenario in all crops. Exposure of mammals will be predominantly dietary, through the consumption of residues on food items. Direct exposure of mammals to NUTREL applications is considered unlikely, since at the time of application and for a short period thereafter, most mammals will leave the immediate vicinity of spray operations in response to the human disturbance.

The proposed use of NUTREL as a insect attractant in orchards implies that the vegetation present on these surfaces that are treated with the product may potentially be consumed by herbivores. The appropriate exposure scenarios are thus deemed to be orchards. The recommended scenario for orchards at the screnning step is that of a small herbivorous mammal with short-cut values of 136.4 and 72.3 for acute and long-term assessments respectively.

In theory, the DDD (daily dietary dose) were calculated by multiplying the application rate (kg/ha) by the short-cut value and the MAF (to account for multiple applications). On top of this, for the long term exposure, the result was multiplied by TWA (factor to account for the time weighted average). This is summarized in the following equations:

DDD_{acute} = Application rate (kg a.s./ha) × short-cut value × MAF
DDD_{repro} = Application rate (kg a.s./ha) × short-cut value × MAF × TWA

Where:

- the short-cut value is given by the guidance document
- MAF is the Multiple Application Factor. It is not relevant as the use of NUTREL involves only one application.
- The term TWA is the time-weighted-average factor. This was used to calculate time-weighted average (TWA) residues on leafy crops, which take into account the degradation of the active substances over time. TWA residues were used as an estimate of long-term exposure only, since it is considered that the use of maximum residues provides an unrealistically extreme worst-case estimate of long-term exposure. A TWA of 1 was used as given in the Guidance Document, assuming no degradation of the active substance over time.

The resulting daily dietary doses are presented in the table 10.3-4.

Table 10.1.2-4: Daily dietary doses for Hydrolysed proteins at the screening step for 2,4 L/ha (all crops)

Test substance	Crop, use pattern	Indicator species	Assessment type	Short-cut value	DDD (mg/kg bw/day)
Hydrolysed proteins	Orchards	Small herbivorous mammal (screening step)	Acute	N.A	N.A
			Reproduction	N.A	N.A

Since the screening step for the reproductive dietary risk was unacceptable, the tier 1 assessment was triggered by using the generic focal species into account:
-Large herbivorous mammal “lagomorph”, Non-grass herbs 100%, Non-grass herbs with a short-cut value: No data available*.
- Small herbivorous mammal "vole Grass + cereals 100% grass with a short-cut value: No data available*.
- Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds , 50% weed seeds , 25% ground arthropods with a short-cut value No data available*.

The new reproductive daily dietary doses for Hydrolysed proteins are presented in the table 10.3-5 below.

Table 10.1.2-5: Daily dietary doses for Hydrolysed proteins for the tier 1

Test substance	Crop, use pattern	Indicator species	Assessment type	Short-cut value	TWA	DDD (mg/kg bw/day)
Hydrolysed proteins	Orchard application, crop directed >BBCH 71	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	Reproduction	N.A	1	N.A
		Small herbivorous mammal "vole Grass + cereals 100% grass	Reproduction	N.A	1	N.A
		Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	Reproduction	N.A	1	N.A

CP 10.1.2.2 Higher tier data on mammals
Toxicity exposure ratios
Acute toxicity exposure ratio (TER_A)

In theory, the acute risk to wild mammals was assessed by calculation of toxicity exposure ratios (TER_A) according to the following equation:

$$TER_A = \frac{LD_{50}\,(mg/kg\,bw/day)}{Acute\,DDD\,(mg/kg\,bw/day)}$$

Acute risk was calculated using the lowest acute LD_{50} values for the active substance. According to the Annex VI guidelines, a TER_A value below 10 indicates a potential acute risk to mammals. The results are presented in Table 10.3.1.1-1.

Table 10.3.1.1-1: Acute risk (TER_A) to mammals (to 2 s.f.) from Hydrolysed proteins in all crops

Compound	Scenario	Indicator species	App. rate (g a.s./ha)	LD_{50} (mg a.s./kg bw/day)	Acute DDD (mg a.s./kg bw/day)	TER_A
Hydrolysed proteins	Orchards	Small herbivorous mammal (screening step)	500-1000	N.A.	N.A.	N.A.

Conclusion: The TER_A value is greater than the Annex VI trigger of 10, indicating low acute risk to mammals from Hydrolysed proteins following application of NUTREL in all crops. This result can be extrapolated to apples which requires a lower rate than pears.

Review Comments: IIIA 10.1.2.2/01	
Agreed endpoints: IIIA 10.1.2.2	

CP 10.1.2.2.1 Short-term toxicity exposure ratio (TER_{ST})

According to the EFSA guidance document, short-term risk to mammals is not presented as it is covered by the long-term risk assessment.

CP 10.1.2.2.2 Long-term toxicity exposure ratio (TER_{LT})

In theory, the long-term risk to wild mammals was assessed from long-term TER values, calculated according to the following equation:

$$TER_{LT} = \frac{NOEC\,(mg/kg\ bw/day)}{Long\text{-}term\ DDD\,(mg/kg\ bw/day)}$$

The lowest NOEL value for Hydrolysed proteins (Table 10.3-3) was used to calculate the TER values in order to provide a worst-case scenario. The resulting TER_{LT} values are given in Table 10.3.1.3-1.

Table 10.1.2.2.2-1: Reproductive risk (TER_{LT}) to mammals from Hydrolysed proteins

Compound	Scenario	Indicator species	App. rate (g a.s./ha)	NOAEL (mg a.s./kg bw/day)	Repro DDD (mg a.s./kg bw/day)	TER_{LT}
Hydrolysed proteins	Orchard	Small herbivorous mammal (screening step)	500-1000	N.A.	N.A.	N.A.

TERs shown in bold fall below the relevant trigger

Based on the screening step, the use of NUTREL gives unacceptable reproductive risk to mammals. Refinement was done by using the generic focal species (see point 10.3 for more details). The results are presented in table 10.3.1.3-2 and show the risk to mammals is acceptable.

Table 10.1.2.2.2-2: Reproductive risk (TER_{LT}) to mammals from Hydrolysed proteins based on the tier 1 assessment

Compound	Scenario	Indicator species	App. rate (g a.s./ha)	NOAEL (mg a.s./kg bw/day)	Repro DDD (mg a.s./kg bw/day)	TER_{LT}
Hydrolysed proteins	Orchard	Large herbivorous mammal “lagomorph” Non-grass herbs 100% Non-grass herbs	500-1000	N.A.	N.A.	N.A.
		Small herbivorous mammal "vole Grass + cereals 100% grass	500-1000	N.A.	N.A.	N.A.
		Small omnivorous mammal “mouse” Combination (invertebrates without interception) 25% weeds 50% weed seeds 25% ground arthropods	500-1000	N.A.	N.A.	N.A.

Review Comments: IIIA 10.1.2.2.2/01	
Agreed endpoints: IIIA 10.1.2.2.2	

CP 10.1.3 Effects on other terrestrial vertebrate wildlife (reptiles and amphibians)
Effects on terrestrial vertebrates other than birds

CP 10.1.3.1 Acute oral toxicity of the preparation

The acute oral toxicity study with NUTREL on rats can be found in Part B, Section 3 Toxicology (point IIIA 7.1.1). For the chronic toxicity, no mammalian toxicity tests with the formulation were performed, since it is possible to extrapolate from data obtained with the active substance.

CP 10.1.3.2 Acceptance of bait, granules or treated seed (palatability testing)

No study on palatability with the formulation was performed as this is no data requirement for products that are to be applied by downward spraying in the field.

Study Comments: IIIA 10.1.3.2/01	
Agreed endpoint/s: IIIA 10.1.3.2	

CP 10.1.3.3 Effects of secondary poisoning

According to the 'Guidance of EFSA – Risk assessment for Birds and Mammals' (EFSA 2009), substances with a log P_{OW} greater than 3 have potential for bioaccumulation and should be assessed for the risk of biomagnification in terrestrial food chains.

In the case of Hydrolysed proteins, this is not relevant since it has a log P_{OW} of 2.16 at pH 7. For this reason, no risk of biomagnification in terrestrial food chain is expected following the application of NUTREL in apple or pear orchards.

Study Comments: IIIA 10.1.3.3/01	
Agreed endpoint/s: IIIA 10.1.3.3	

CP 10.1.3.4 Supervised cage or field trials

Supervised cage/field trials with the formulation were not performed, since low risk to mammals indicates that further studies are not required (TER_A and TER_{LT} values were higher than 10 and 5 respectively).

CP 10.2 Effects on Aquatic Organisms

Risk assessment for aquatic organisms

CP 10.2.1 Acute toxicity to fish, aquatic invertebrates, or effects on aquatic algae and macrophytes

CP 10.2.2 Additional long-term and chronic toxicity studies on fish, aquatic invertebrates and sediment dwelling organisms

CP 10.2.3 Further testing on aquatic organisms

CP 10.2 Effects on Aquatic Organisms

Effects on Aquatic organisms for NUTREL were not evaluated as part of the EU review of Hydrolysed proteins. Data are evaluated here and are considered adequate to perform the risk assessments. Risk assessments for NUTREL with the proposed use pattern are also provided in this core assessment. A national addendum might be presented depending on the country.

Toxicity

A summary of the toxicity data for all Aquatic organisms used in the risk assessment is provided in Table 10.2-1.

The acute toxicity of NUTREL to fish, daphnia, algae and *Lemna* not was determined from studies performed with NUTREL which, therefore, were not evaluated as part of the EU review of the Hydrolysed proteins. Further details regarding the tests with the product NUTREL are provided in section 10.2-3 whilst details of the studies on the active substance are provided in the Hydrolysed proteins EU review.

Table 10.2-1: Summary of the toxicity values of Hydrolysed proteins for aquatic organisms

Organism	Test substance	Endpoint	Value	Reference
Fish				
Brachydanio rerio	Hydrolysed proteins	96h LC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Rainbow trout *Oncorhynchus mykiss*	NUTREL	96h LC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Aquatic invertebrates				
Daphnia magna	Hydrolysed proteins	48h EC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
	NUTREL		mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
	Hydrolysed proteins	21-day NOEC	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Algae				
Pseudokirchneriella subcapitata (= Selenastrum capricornutum)	Hydrolysed proteins	72h E_bC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
	NUTREL	72h E_bC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Anabaena flos-aquae	NUTREL	72h E_bC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Navicula pelliculosa	Hydrolysed proteins	72h E_bC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Aquatic macrophytes				
Lemna gibba	Hydrolysed proteins	7-day EC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Lemna minor	NUTREL	7-day EC_{50}	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***
Sediment dwelling organisms				
Chironomus riparius	Hydrolysed proteins	28d NOEC	mg a.i./L No studies were submitted	***DAR (2008)*** ***EFSA (2012)***

The EFSA accepts the argumentation that the active substances derived from the hydrolysis of animal tissues do not have any significant toxicity potential.
Tests with gastropod molluscs and insects other than sediment dwellers are not required. Two or four applications are recommended so no continued and NUTREL is not intended for direct application in water bodies.

Metabolites of Hydrolysed proteins

Not relevant since there are no relevant metabolites of Hydrolysed proteins in water.

Exposure

Aquatic organisms may be exposed to NUTREL and Hydrolysed proteins through spray drift, run-off and drainage from the application site into adjacent water bodies. In theory, exposure of aquatic organisms from these routes was estimated by calculating Predicted Environmental Concentrations in surface water (PEC_{SW}) (see Part B, Section 5).

Hydrolysed proteins

PEC values in surface water for Hydrolysed proteins: not data submitted. PEC values in surface water for Hydrolysed proteins not were calculated using the FOCUS surface water models following 2-4 applications of NUTREL (378 g/L) at 2.4 L product/ha. FOCUS Step 1 PECSW values were calculated theoretically using an extreme worst-case exposure scenario. Further FOCUS Step 2 PECSW values were calculated theoretically .For full details of the assumptions used in the exposure calculations, see Part B, Section 5.

The resulting worst-case FOCUS Step 2 PEC_{SW} values for Hydrolysed proteins are presented in Table 10.2-2.

Table 10.2-2:FOCUS step 2 PEC_{SW} values for Hydrolysed proteins following 2-4 applications NUTREL

Test substance	Crop	PEC_{SW} (µg/L)
Hydrolysed proteins	All crop	N.A.
Hydrolysed proteins	All crop	N.A.

CP 10.2.1 Toxicity exposure ratios

The NUTREL and Hydrolysed proteins risk assessments not were carried out following application according to the proposed use.

In theory, the initial risk assessments were carried out by comparing the PEC_{SW} values with the acute and long-term toxicity endpoints. Acute and long-term toxicity exposure ratios (TER_A and TER_{LT}) were calculated using the following equations:

$$TER_A = \frac{EC_{50} / LC_{50}}{\text{Maximum } PEC_{SW}}$$

$$TER_{LT} = \frac{NOEC}{\text{Maximum } PEC_{SW} / 21\,\text{d TWA } PEC_{SW}}$$

CP 10.2.1.1 TER_A for Fish

In theory, the acute toxicity endpoint for NUTREL on Rainbow trout was used in the risk assessment since it is the lowest value. The resulting acute TER value, based on the maximum instantaneous PEC_{SW} value, following two-four applications to all crop, at 3 m from the application site (default STEP 2 distance) is shown below.

Table 10.2.1.1-1 Fish acute TER value for NUTREL in all crops

Crop	Test organism	Test substance	96 hr LC_{50} (µg a.i./L)	PEC_{SW} (µg a.i./L)	TER_A	Trigger value
All crops	Rainbow trout	NUTREL	N.A.	N.A.	N.A.	100

The TER for Hydrolysed proteins is above the Annex VI trigger value of 100, indicating that NUTREL poses low acute risk to fish.

Review Comments: IIIA 10.2.1.1	
Agreed endpoints: IIIA 10.2.1.1	

CP 10.2.1.2 TER_{LT} for Fish

NUTREL will not persist in natural water. Hydrolysed proteins degrades rapidly in the water/sediment system (DT_{50} of days in the water and days in the whole system, not are required, because the product is constituted by natural substances and is completely biodegradable).

Moreover, the GAP recommend two-four applications at 10-30 days, so that the continuous or repeated exposure to the formulation and to the active substance is unlikely. This is why no chronic toxicity study with fish was found to be necessary during the EU review. It is concluded that NUTREL poses low chronic risk to fish.

CP 10.2.1.3 TER_A for Daphnia

In theory, the acute toxicity endpoint for NUTREL on Daphnia was used in the risk assessment since it is the lowest value. The resulting acute TER value, based on the maximum instantaneous PEC_{SW} value, following two-four applications to all crop, at 3 m from the application site (default STEP 2 distance) is shown below.

Table 10.2.1.3-1 Daphnia magna acute TER value for NUTREL in all crops

Crop	Test organism	Test substance	96 hr LC_{50} (µg a.i./L)	PEC_{SW} (µg a.i./L)	TER_A	Trigger value
All crops	*Daphnia magna*	NUTREL	N.A.	N.A.	N.A.	100

The TER for Hydrolysed proteins is above the Annex VI trigger value of 100, indicating that NUTREL poses low acute risk to *Daphnia magna*.

Review Comments: IIIA 10.2.1.3	
Agreed endpoints: IIIA 10.2.1.3	

CP 10.2.1.4 TER_{LT} for Daphnia

In theory, the long term NUTREL TER for *Daphnia magna* was calculated using the peak PEC_{SW} at 3 m from the application site (default STEP 2 distance) following two-four applications of NUTREL to all crops. The resulting TER_{LT} is presented below:

Table 10.2.1.4-1: Long-term TER value for *D. magna* for NUTREL

Crop	Test organism	Test substance	21-d NOEC (µg a.i./L)	PEC_{SW} (µg a.i./L)	TER_{LT}	Trigger value
All crops	*Daphnia magna*	Hydrolysed proteins	N.A.	N.A.	N.A.	10

The TER_{LT} value is above the Annex VI trigger of 10, indicating a low risk to *D. magna* following application of NUTREL.

Review Comments: IIIA 10.2.1.4	
Agreed endpoints: IIIA 10.2.1.4	

CP 10.2.1.5 TER_A for Aquatic insect

In theory, TER_A values for Aquatic insects are not required since the risk assessments for *Daphnia magna* (Point 10.2.1.3) indicated that NUTREL poses low acute risk to aquatic invertebrates. In addition, this data point is not relevant since NUTREL is not intended for use directly on surface waters.

Review Comments: IIIA 10.2.1.5	
Agreed endpoints: IIIA 10.2.1.5	

CP 10.2.1.6 TER_{LT} for Aquatic insect

In theory, the long term NUTREL TER for Aquatic insect was calculated using the peak PEC_{SW} at 3 m from the application site (default STEP 2 distance) following two-four applications of NUTREL to all crops. The resulting TER_{LT} is presented below:

Table 10.2.1.6-1: Long-term TER value for *Chironomus riparius* for NUTREL

Crop	Test organism	Test substance	28-d NOEC (µg a.i./L)	PEC_{SW} (µg a.i./L)	TER_{LT}	Trigger value
All crops	*Chironomus riparius*	Hydrolysed proteins	N.A.	N.A.	N.A.	10

The TER_{LT} value is above the Annex VI trigger of 10, indicating a low risk to *Chironomus riparius* following application of NUTREL.

Review Comments: IIIA 10.2.1.6	
Agreed endpoints: IIIA 10.2.1.6	

CP 10.2.1.7 TER_A for Aquatic crustacean

In theory, TER_A values for additional Aquatic crustacean species are not required since the risk assessments for *Daphnia magna* (Point 10.2.1.3) indicated that NUTREL poses low acute risk to aquatic invertebrates.

Review Comments: IIIA 10.2.1.7	
Agreed endpoints: IIIA 10.2.1.7	

CP 10.2.1.8 TER_{LT} for Aquatic crustacean

In theory, TER_{LT} values for additional Aquatic crustacean species are not required since the risk assessments for *Daphnia magna* (Point 10.2.1.4) indicated that NUTREL pose low long-term risk to aquatic invertebrates.

Review Comments: IIIA 10.2.1.8	
Agreed endpoints: IIIA 10.2.1.8	

CP 10.2.1.9 TER_A for Aquatic gastropod molluscs

In theory, TER_A values for Aquatic gastropod molluscs are not required since the risk assessments for *Daphnia magna* (Point 10.2.1.3) indicated that NUTREL poses low acute risk to aquatic invertebrates. In addition, this data point is not relevant since NUTREL is not intended for use directly on surface waters.

Review Comments: IIIA 10.2.1.9	
Agreed endpoints: IIIA 10.2.1.9	

CP 10.2.1.10 TER_{LT} for Aquatic gastropod mollusc

In theory, TER_{LT} values for Aquatic gastropod molluscs are not required since the risk assessments for *Daphnia magna* (Point 10.2.1.4) indicated that NUTREL poses low long-term risk to aquatic invertebrates. In addition, this data point is not relevant since NUTREL is not intended for use directly on surface waters.

Review Comments: IIIA 10.2.1.10	
Agreed endpoints: IIIA 10.2.1.10	

CP 10.2.1.11 TER_{LT} for Algae

In theory, the long-term risk to Algae from NUTREL was assessed using the E_bC_{50} value for *Anabaena flos-aquae* (Table 10.2-1) since this was the lowest EC_{50}, and therefore provided a worst case scenario. The resulting TER, calculated using the maximum instantaneous PEC_{SW} value following two-four applications at 3m from the application site, is given in the Table 10.2.1.11-1.

Table 10.2.1.11-1 Algae TER_{LT} value for NUTREL

Crop	Test organism	Test substance	96 hr E_bC_{50} (µg a.i./L)	PEC_{SW} (µg a.i./L)	TER	Trigger value
All crops	*Anabaena flos-aquae*	Hydrolysed proteins	N.A.	N.A.	N.A.	10

The TER for NUTREL is above the Annex VI trigger value of 10, indicating that application of NUTREL according to the proposed label uses poses low risk to algae.

Review Comments: IIIA 10.2.1.11	
Agreed endpoints: IIIA 10.2.1.11	

CP 10.2.1.12 TER for Aquatic plants

In theory, the long-term Toxicity Exposure ratios (TER_{LT}), calculated at different distances from the treated area are given, to significant figures, in Table 10.2.1.12-1. The lowest toxicity value (from the theoretically study on Hydrolysed proteins) was used.

Table 10.2.1.12-1: Risk to aquatic macrophytes

Crop	Test organism	Test substance	EC_{50} (µg a.i./L)	PEC_{SW} (µg a.i./L)	TER	Trigger value
All crops	*Lemna gibba*	Hydrolysed proteins	N.A.	N.A.	N.A.	10

TERs shown in bold fall below the relevant trigger

In theory, the TER values calculated for the worst case of exposure at 3 m distance from the application area (default STEP 2 distance) exceed the 91/414/EEC Annex VI trigger value of 10, indicating a low risk to aquatic plants.

Review Comments: IIIA 10.2.1.12	
Agreed endpoints: IIIA 10.2.1.12	

CP 10.2.2 Acute toxicity of the formulation

CP 10.2.2.1 Fish

No study were submitted.

The following fish acute toxicity study performed on NUTREL not is provided in support of the assessment and the only evaluation on EU level, is reported in the DAR 2008 and EFSA 2012.

Report:	No data available*
Title:	The acute toxicity of NUTREL to rainbow trout (*Onchorynchus mykiss*) over a 96 hour exposure period.
Document No:	Data gap
Guidelines:	OECD 203
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Materials and methods
Data gap

Observations
Data gap

Table 10.2.2.1-1 Findings for toxicity to rainbow trout

Test substance	NUTREL
Test object	Rainbow trout
Exposure	96 h, semi-static
LC_{50} mg product/L	No data available*
LOEC	No data available*
NOEC	No data available*
LC_{50} mg a.i./L *	No data available*

Conclusions
Data gap

Study Comments: IIIA 10.2.2.1/01	
Agreed endpoint/s: IIIA 10.2.2.1	

CP 10.2.2.2 Aquatic invertebrates (Daphnia)

No study were submitted.

The following aquatic invertebrate toxicity study performed on NUTREL not is provided in support of the assessment and the only evaluation on EU level, is reported in the DAR 2008 and EFSA 2012.

Report:	No data available*
Title:	The toxicity to *Daphnia magna* of NUTREL
Document No:	Data gap
Guidelines:	OECD 202
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Materials and methods
Data gap

Observations
Data gap

Table 10.2.2.2-1 Findings for toxicity to *Daphnia magna*

Test substance	NUTREL
Test object	*Daphnia magna*
Exposure	48 h, semi-static
EC_{50} mg product/L	No data available*
NOEC	No data available*
EC_{50} mg a.i./L*	No data available*

Conclusions
Data gap

Study Comments: IIIA 10.2.2.2/01	
Agreed endpoint/s: IIIA 10.2.2.2	

CP 10.2.2.3 Algae

No study were submitted.

The following algae toxicity study performed on NUTREL not is provided in support of the assessment and the only evaluation on EU level, is reported in the DAR 2008 and EFSA 2012.

Report:	No data available*
Title:	The growth inhibition of the alga *Pseudokirchneriella subcapitata* by NUTREL
Document No:	Data gap
Guidelines:	OECD 201
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Materials and methods
Data gap

Observations
Data gap

Table 10.2.2.3-1 Findings for toxicity to algae

Test substance	NUTREL
Test object	*Pseudokirchneriella subcapitata*
Exposure	72 h, static
E_bC_{50} mg product/L (biomass) E_rC_{50} mg product/L (growth rate)	No data available* No data available*
NOE_bC	No data available*
E_bC_{50} mg a.i./L (biomass) E_rC_{50} mg a.i./L (growth rate)	No data available* No data available*

Conclusion:
Data gap

Report:	No data available*
Title:	The growth inhibition of NUTREL to the alga *Anabaena flos-aquae* over a 72 hour exposure period
Document No:	Data gap
Guidelines:	OECD 201
GLP	Yes

Materials and methods
Data gap

Observations
Data gap

Table 10.2.2.3-2 Findings for toxicity to algae

Test substance	NUTREL
Test object	*Anabaena flos-aquae*
Exposure	72 h, static
E_bC_{50} mg product/L (biomass) E_rC_{50} mg product/L (growth rate)	No data available* No data available*
NOE_bC	No data available*
E_bC_{50} mg a.i./L (biomass) E_rC_{50} mg a.i./L (growth rate)	No data available* No data available*

Conclusion:
Data gap

Study Comments: IIIA 10.2.2.3/01	
Agreed endpoint/s: IIIA 10.2.2.3	

CP 10.2.2.4 Marine or estuarine organisms

This is not an EC data requirement / not required by Directive 91/414/EEC.

CP 10.2.2.5 Marine sediment invertebrates

This is not an EC data requirement / not required by Directive 91/414/EEC.

CP 10.2.3 Microcosm or mesocosm study

Not deemed necessary since the risk is acceptable was found to be acceptable on lower tier.

CP 10.2.4 Residue data in fish

Not deemed necessary since the risk is acceptable was found to be acceptable on lower tier.

CP 10.2.5 Chronic toxicity to fish

NUTREL will not persist in natural water. Hydrolysed proteins degrades rapidly in the water/sediment system. This is why no chronic toxicity study with fish was found to be necessary during the EU review. It is concluded that NUTREL poses low chronic risk to fish.

CP 10.2.6 Chronic toxicity to aquatic invertebrates

As risk assessments indicate that the active substance of NUTREL does not pose an unacceptable chronic risk to aquatic invertebrates, tests with NUTREL are not required.

CP 10.2.7 Accumulation in aquatic non-target organisms

Bioaccumulation of any of the active substances under natural conditions is not expected to occur (refer to Section 10.2.4), and a study is not necessary to determine bioaccumulation in aquatic non-target organisms.

Study Comments: IIIA 10.2.7/01	
Agreed endpoint/s: IIIA 10.2.7	

CP 10.2.3 Further testing on aquatic organisms

No data available.

CP 10.3 Effects on Arthropods

CP 10.3.1 Effects on bees

Risk assessment for bees

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

CP 10.3.1.1.2 Acute contact toxicity to bees

CP 10.3.1.2 Chronic toxicity to bees

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages

CP 10.3.1.4 Sub-lethal effects

CP 10.3.1.5 Cage and tunnel tests

CP 10.3.1.6 Field tests with honeybees

CP 10.3.1 Effects on Bees

EU Endpoints: Effects on Bees

Ecotoxicological endpoints for bees

Active substance	EU agreed endpoints (EFSA Journal 2012;10(2):2545)	Remark
Hydrolysed proteins	Oral (48 h) LD_{50} (µg/bee) Contact (48 h) LD_{50} (µg/bee)	No data available* No data available*

Summary

Effects on bees of NUTREL were not evaluated as part of the EU review of Hydrolysed proteins. Therefore all relevant data and assessments are provided here and are considered adequate.

Testing for effects of NUTREL on bees not was carried out using the formulated product. In theory, both acute oral and acute contact toxicity were tested leading to oral and contact LD_{50} of respectively > xx and >x µg a.i./bee. The risk assessment is provided for all crops in the table 10.4-1 using the EU agreed endpoints.

Table 10.3.1-1 Hazard quotients for honey bees in all crops

Test substance	Use pattern	Exposure route	Endpoint	Maximum single application rate	Hazard quotient (HQ)	HQ assessment trigger
Hydrolysed proteins	Air-assisted spraying	Contact	LD_{50} (µg ai/bee) N.A.	1000 g/ha	N.A.	N.A.
Hydrolysed proteins	Air-assisted spraying	Oral	LD_{50} (µg ai/bee) N.A.	1000 g/ha	N.A.	N.A.

Toxicity

Table 10.3.1-2 presents the results of bee toxicity studies. Further details regarding the tests with the formulation NUTREL are provided in section 10.4.2.

Table 10.3.1-2: Toxicity to bees of NUTREL

Substance	Endpoint	Value	Reference
NUTREL	48 h contact LD_{50}	(> x µg ai/bee) N.A.	***DAR (2008)***
	48 h oral LD_{50}	(> xx µg ai/bee) N.A.	

Exposure

Applications of pesticides can potentially result in exposure of honeybees either through direct over-spray, or by contact with residues on plants whilst bees are foraging for food. Risk assessment is conducted in order to consider an extreme worst-case scenario at the maximum application rates for NUTREL.
Overall the proposed use of Hydrolysed proteins is considered to pose a low risk to bees. No further data are required.

CP 10.3.1.1 Acute toxicity to bees
Hazard quotients for bees

No study were submitted.

In theory, the acute risk to honeybees from use of NUTREL was assessed using the maximum single application rate and the lowest LD_{50} values (from the active substance) to calculate hazard quotients ***(EPPO 2003)***[2] as follows:

$$Hazard\ Quotient = \frac{Maximum\ application\ rate\ (g\ formulation/ha)}{Acute\ LD_{50}\ (\mu g\ formulation/bee)}$$

Hazard quotients were calculated for oral exposure (Q_{HO}) and contact exposure (Q_{HC}) to NUTREL. A hazard quotient of less than 50 indicates a low risk to bees in the field.

Table 10.3.1.1-1: Risk to bees from exposure to NUTREL in all crops

Test substance	Application rate (g a.i./ha)	LD_{50} (µg/bee)	Hazard quotient
Hydrolysed proteins	1000	Contact LD_{50} (>xx µg ai/bee) N.A.	(< x) N.A.
		Oral LD_{50} (> x µg ai/bee) N.A.	(< xx) N.A.

[2] EPPO/OEPP (2003) Environmental risk assessment scheme for plant protection products, Chapter 10: Honeybees (PP 3/10(2)). Bulletin OEPP/EPPO Bulletin 33: 141-145.

All the hazard quotients are considerably less than 50 (> x and > xx) indicating that the active substance poses a low risk to bees. Therefore a low risk to bees is expected from the application of NUTREL in all crops.

Comments: IIIA 10.3.1.1/01	
Agreed endpoint/s: IIIA 10.3.1.1	

CP 10.3.1.1.1 Acute oral toxicity to bees
Acute toxicity of the formulation to bees

Oral

No study were submitted.
The following bee acute and contact toxicity study performed on NUTREL not is provided in support of the assessment and the only evaluation on EU level, is reported in the DAR 2008 and EFSA 2012.

Report:	No data available*
Title:	Acute contact and oral toxicity of Hydrolysed proteins on honey bees (*Apis mellifera*)
Document No:	Data gap
Guidelines:	OECD 213 and 214
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Materials and methods
Data gap

Observations
Data gap

Summary
The mortality data are presented in the table 10.3.2.1-1. The mortality in the control groups in both of the oral and contact studies was at the accepted level (≤10 %). No adverse effect was observed on the behaviour.

CP 10.3.1.1.2 Acute contact toxicity to bees
Contact exposure Q_{HC}

Reference is made to Point 10.3.1.1 above.

Table 10.3.1.1-1: Bee mortality data for NUTREL

Oral study (48 h)

	Control (sucrose solution)	Nominal dose µ Formulated product/bee				
Cumulative mortality	/	//////////	//////////	//////////	//////////	//////////
Percentage mortality (%)	/	//////////	//////////	//////////	//////////	//////////

Study Comments: IIIA 10.3.2.1/01	
Agreed endpoint/s: IIIA 10.3.2.1	

Contact study (48 h)

	Control (deionised water)	Control (acetone solution)	Nominal dose µ Formulated product/bee
Cumulative mortality	/		//////////
Percentage mortality (%)	/		//////////

CP 10.3.1.2 Chronic toxicity to bees

As NUTREL does not pose an unacceptable risk to honey-bees, further tests are not necessary ($Q_{HC} < 50$).

CP 10.3.1.3 Effects on honey bee development and other honey bee life stages Investigation into special effects

As NUTREL does not pose an unacceptable risk to honey-bees, further tests are not necessary ($Q_{HC} < 50$).

CP 10.3.1.4 Sub-lethal effects - Effects on bees of residues on crops

As NUTREL does not pose an unacceptable risk to honey-bees, further tests are not necessary ($Q_{HC} < 50$).

CP 10.3.1.5 Cage and tunnel tests

As NUTREL does not pose an unacceptable risk to honey-bees, further tests are not necessary ($Q_{HC} < 50$).

CP 10.3.1.6 Field tests with honeybees

As NUTREL does not pose an unacceptable risk to honey-bees, further tests are not necessary ($Q_{HC} < 50$).

CP 10.3.2 Effects on non-target arthropods other than bees

Risk assessment for other non-target arthropods

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

CP 10.3.2.2 Extended laboratory testing, aged residue studies with non-target arthropods

CP 10.3.2.3 Semi-field studies with non-target arthropods

CP 10.3.2.4 Field studies with non-target arthropods

CP 10.3.3 Other routes of exposure for non-target arthropods

CP 10.3.2 Effects on Arthropods Other Than Bees

No study were submitted.

The following Arthropods Other Than Bees toxicity study performed on NUTREL not is provided in support of the assessment and the only evaluation on EU level, is reported in the DAR 2008 and EFSA 2012.

EU Endpoints: Effects on Arthropods
Ecotoxicological endpoints for Arthropods

Active substance	EU agreed endpoints (EFSA Journal 2012; 10(2):2545)	Endpoints used in risk assessment
Acute		
Hydrolysed proteins	*Typhlodromus pyri* LC_{50} (> x g a.i./ha)	
Hydrolysed proteins		*Typhlodromus pyri* LC_{50} (g a.i./ha) N.A.
Hydrolysed proteins	*Aphidius rhopalosiphi* LC_{50} (> x g a.i./ha)	
Hydrolysed proteins		*Aphidius rhopalosiphi* LC_{50} (g a.i./ha) N.A.
Hydrolysed proteins	*Chrysoperla carnea* LC_{50} (> x g a.i./ha)	
Hydrolysed proteins		*Chrysoperla carnea* LC_{50} (g a.i./ha) N.A.
Hydrolysed proteins		*Orius laevigatus* LC_{50} (g a.i./ha) N.A.

Summary

Effects on arthropods other than bees of NUTREL were not evaluated as part of the EU review of Hydrolysed proteins. Therefore all relevant data and assessments are provided here and are considered adequate.

Toxicity

The toxicity of NUTREL to non-target arthropods has been investigated. The testing and risk assessment strategy used here follow the approach recommended in the *ESCORT 2 guidance document* (*Candolfi et al. 2001*)[3] and the *EC Guidance Document on Terrestrial Ecotoxicology* [4].

[3] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[4] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

Exposure

In-field

Non-target arthropods living in the crop can be exposed to residues from NUTREL by direct contact either as a result of overspray or through contact with residues on plants and soil or in food items.

NUTREL 297 g/L is applied at a maximum rate of 1 x 3,050 L formulation/ha (906 g a.i./ha) in all crops;
NUTREL 300 g/L is applied at a maximum rate of 1 x 3,0 L formulation/ha (900 g a.i./ha) in all crops;
NUTREL 421 g/L is applied at a maximum rate of 1 x 2,140 L formulation/ha (901 g a.i./ha) in all crops;
NUTREL 378 g/L is applied at a maximum rate of 1 x 2,4 L formulation/ha (907 g a.i./ha) in all crops;

Even though the product is applied at BBCH 71-79, the crop interception will be considered as 100% for foliar dwelling organisms.

The maximum in-field exposure (Predicted Environmental Rate, PER) to foliar-dwelling organisms amounts to 1000 g a.i./ha. For the soil-dwelling organisms, a 0% crop interception will be considered and therefore the maximum in-field exposure (Predicted Environmental Rate, PER) for these organisms amounts to 1000 g a.i./ha.

In theory, the in-field exposure (predicted environmental residue, PER) is calculated according to ESCORT 2 using the following equation:

$$\mathrm{PER}_{\mathrm{in-field}} = \text{Application rate (g ai/ha)} \times \mathrm{MAF}$$

The MAF is a generic multiple application factor, which is used to take into account the potential build-up of applied substances between applications based on the application interval, DT_{50} value and number of applications. Default foliar and soil MAF values following several applications are given in the ESCORT 2 Guidance Document. Since 6-BA 100 SL is applied only once a season, the multiple application factor MAF, can be omitted (MAF = 1).

The maximum predicted environmental residues (PER) occurring within the field after application of NUTREL at the maximum application rate are presented in Table 10.3.2-1.

Table 10.3.2-1: In-field PER values for application of NUTREL

Crop	Substance	Application rate	PER (foliar)	PER (soil)
All crops	NUTREL	1000 g a.i./ha	1000 g a.i./ha	1000 g a.i./ha

Off-field

Risk assessment of areas immediately surrounding the crop is considered important since these areas represent a natural reservoir for immigration, emigration and reproduction of arthropod populations and provide increased species diversity. Exposure of non-target arthropods living in off-field areas to NUTREL will mainly be due to spray drift from field applications. Off-field areas are assumed to be densely vegetated and thus spray drift is unlikely to reach bare ground.

Therefore, evaluation of exposure *via* soil residues in off-field areas was not considered. In theory, off-field foliar PER values were calculated from in-field foliar PERs in conjunction with drift values published by the *BBA (2000)*[5] as shown in the following equation:

$$\text{Off - field foliar PER} = \frac{\text{Maximum in - field foliar PER x (\% drift/100)}}{\text{vegetation distribution factor}}$$

Vegetation distribution factor: In theory, the model used to estimate spray drift was developed for drift onto a two-dimensional water surface and, as such, does not account for interception and dilution by three-dimensional vegetation in off-crop areas. Therefore, a vegetation distribution or dilution factor is incorporated into the equation when calculating PERs to be used in conjunction with toxicity endpoints derived from two-dimensional (glass plate or leaf disc) studies. A dilution factor of 10 is recommended by ESCORT 2. For 3-dimensional studies, i.e. where spray treatment is applied onto whole plants, the dilution factor of 10 is not used, as any dilution over the 3-dimensional vegetation surface is accounted for in the study design.

The drift value at 3 m distance is 15.73% of the application rate (90th percentile drift). The drift factor (% drift/100) is therefore 15.73/100 = 0.1573.

The resulting PERoff-field values are shown in Table 10.3.2-2.

Table 10.3.2-2**: Off-field foliar Predicted Environmental Rates (PER)**

Study type	**Maximum in-field foliar PER** [a]	**Drift factor (% drift/100)**	**Vegetation distribution factor**	**Off-field foliar PER L/ha (g a.i./ha)**
Glass plate	1000 g a.i./ha	N.A.	10	N.A.
Plants	1000 g a.i./ha	N.A.	1	N.A.

[a] See Table 10.3.2-1

[5] 90th percentile drift according to BBA (2000): Bundesanzeiger Jg. 52 (Official Gazette), Nr 100, S. 9879-9880 (25.05.2000) Bekanntmachung über die Abtrifteckwerte, die bei der Prüfung und Zulassung von Pflanzenschutzmitteln herangezogen werden

Risk assessment

The risk to non-target arthropods is assessed using the approach recommended in the published *ESCORT 2 document (Candolfi et al. 2001)*[6] and the *EC Guidance Document on Terrestrial Ecotoxicology* [7].

In-field

In theory, the potential risk of NUTREL to in-field non-target arthropods was assessed by calculation of the hazard quotient (HQ = exposure/toxicity) with the predicted environmental rate (PER) and the lowest lethal rate (LR_{50}) values according to the following formula:

$$\text{In field HQ} = \frac{\text{In - field PER}}{LR_{50}}$$

The HQ trigger for Tier I laboratory and Tier II extended laboratory studies is 2 and 1, respectively. The resulting $HQ_{in\text{-}field}$ values are presented in Table 10.3.2-2.

Table 10.3.2-2**: In-field HQs for non-target arthropods (calculated for all crops)**

Crop	**Species**	**LR_{50} (g a.i./ha)**	**In-field foliar**		**In-field soil**		**Trigger value**
			PER (g a.i./ha)	**HQ**	**PER (g a.i./ha))**	**HQ**	
All crops	*Typhlodromus pyri* Tier I, 2D exposure scenario	N.A. (g a.i./ha)	1000 g a.i./ha	N.A.	1000 g a.i./ha	N.A.	2
	Aphidius rhopalosiphi Tier I, 2D exposure scenario	N.A. (g a.i./ha)	1000 g a.i./ha	N.A.	1000 g a.i./ha	N.A.	2
	Aphidius rhopalosiphi Tier II, 3D exposure scenario	N.A. (g a.i./ha)	1000 g a.i./ha	N.A.	1000 g a.i./ha	N.A.	1
	Orius laevigatus Tier I, 2D exposure scenario	N.A. (g a.i./ha)	1000 g a.i./ha	N.A.	1000 g a.i./ha	N.A.	1
	Chrysoperla carnea Tier I, 2D exposure scenario	N.A. (g a.i./ha)	1000 g a.i./ha	N.A.	1000 g a.i./ha	N.A.	1
	Chrysoperla carnea Tier II, 2D exposure scenario	N.A. (g a.i./ha)	1000 g a.i./ha	N.A.	1000 g a.i./ha	N.A.	1

Hence, NUTREL poses low risk to in-field non-target athropods following application according to the proposed use patterns.

[6] Candolfi MP, Barrett KL, Campbell PJ, Forster R, Grandy N, Huet M-C, Lewis G, Oomen PA, Schmuck R, Vogt H (2000) 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods' From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.

[7] EC Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329, 17 October 2002.

Off-field

In theory, in order to assess the potential risk of NUTREL to off-field non-target arthropods, the predicted environmental rate (Table 10.5-3) is compared with the toxicity endpoints according to the following formula:

$$\text{Off - field HQ} = \frac{\text{PER}_{\text{off-field}}\ (\text{g/ha})}{\text{LR}_{50}\ (\text{g/ha})} \times \text{Correction factor}$$

The HQ trigger for Tier I laboratory and Tier II extended laboratory studies is 2 and 1, respectively.

Correction factor: ESCORT 2 recommends that a correction factor of 5 be used when assessing Tier II data, or 10 for Tier I data, to account for extrapolation from testing just 2 representative species, to the species diversity expected in off-crop areas.

$HQ_{off\text{-}field}$ values are given, quoted to 2 significant figures, in Table 10.3.2-3.

Table 10.3.2-3**: Off-field HQ values for non-target arthropods (calculated for all crops)**

Species	**LR_{50} (g a.i./ha)**	**Off-field foliar PER (g a.i./ha)**	**Correction factor**	**Off-field foliar HQ**	**Trigger value**
Typhlodromus pyri Tier I, 2D exposure scenario	N.A. (g a.i./ha)	N.A. (g a.i./ha)	10	N.A.	2
Aphidius rhopalosiphi Tier I, 2D exposure scenario	N.A. (g a.i./ha)	N.A. (g a.i./ha)	10	N.A.	2
Aphidius rhopalosiphi Tier II, 3D exposure scenario	N.A. (g a.i./ha)	N.A. (g a.i./ha)	5	N.A.	1
Orius laevigatus Tier I, 2D exposure scenario	N.A. (g a.i./ha)	N.A. (g a.i./ha)	5	N.A.	1
Chrysoperla carnea Tier I, 2D exposure scenario	N.A. (g a.i./ha)	N.A. (g a.i./ha)	5	N.A.	1
Chrysoperla carnea Tier II, 2D exposure scenario	N.A. (g a.i./ha)	N.A. (g a.i./ha)	5	N.A.	1

The off-field HQ values for *T. pyri*, *A. rhopalosiphi*, *Orius laevigatus* and *Chrysoperla carnea* fall below the trigger values, indicating that NUTREL does not pose an unacceptable risk to non-target arthropods in off-field areas.

Comments: IIIA 10.3.2/01	
Agreed endpoint/s: IIIA 10..3.2	

CP 10.3.2.1 Standard laboratory testing for non-target arthropods Using artificial substrates

The following non-target athropod studies using artificial substrates performed on NUTREL not were provided in support of the assessment.

Report:	No data available*
Title:	Effect of Hydrolysed proteins on the predatory mite *Typhlodromus pyri* in a laboratory trial
Document No:	Data gap
Guidelines:	Not stated but similar to Blümel *et al.* 2000
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Summary
In theory, the study encompassed 5 treatment groups (3 dose rates of the test item, control and dimethoate reference item) with 3 replicates each containing 20 mites (5 replicates were used for the water control). The mites were exposed to dried residues on treated glass plates. Survival of the mites was assessed after 1, 3 and 7 days. For the reproduction assessment, surviving mites from the control and from all test item groups displaying less than 50% corrected mortality were sexed and the number of eggs per female was recorded at days 7, 10, 13 and 14. The temperature was 24.2-25.7°C, the relative humidity was 61-86%, the photoperiod was 16h light/8h dark, the light intensity was 797.5 lux.

The results of the assessments are summarized in the table 10.3.2.1-1 below **(Simulated values)**

Table 10.3.2.1-1: Effects of NUTREL to *Typhlodromus pyri* on artificial substrate

Test product	Observed mortality (%)	Number of eggs per female	Inhibition of reproduction
Control	N.A.	N.A.	N.A.
NUTREL (500 g a.i./ha)	N.A.	N.A.	N.A.
NUTREL (600 g a.i./ha)	N.A.	N.A.	N.A.
NUTREL (1000 g a.i./ha)	N.A.	N.A.	N.A.
Dimethoate	N.A.	Not determined	Not determined

Conclusion
The mortality for *Typhlodromus pyri* in the control groups in both of the oral and contact studies was at the accepted level (≤10 %).

Study Comments: IIIA 10.3.2.1/01	
Agreed endpoint/s: IIIA 10.3.2.1	

CP 10.3.2.2 Extended laboratory studies

The following non-target athropod studies using natural substrates performed on NUTREL not were provided in support of the assessment.

Report:	No data available*
Title:	Side effects of Hydrolysed proteins on the predatory bug *Orius laevigatus* (Fieber) on natural substrate in the laboratory
Document No:	Data gap
Guidelines:	Bakker et al., 2000
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Summary

In theory, the study encompassed 3 treatment groups (1 dose rate of the test item NUTREL, control and dimethoate reference item) with 10 replicates each containing 6 larvae bugs. Only four replicates each containing 6 larvae bugs were used for the toxic standard. The predatory bugs were exposed to dry residues on treated bean leaf discs. 1 to 3 hours after the application, 2nd instar nymphs were introduced in the units and fed with *Ephestia kuehniella* eggs and pollen for 9 days. Units were dismantled at day 9 of exposure when mortality was recorded. Young adults were transferred into breeding cages for a five day period. At this date, females of each group were randomly selected and assessed for fertility performance. They were individually confined on untreated leaf discs for 2 periods of 2 day egg laying. Only the first batch of eggs was assessed for hatching.The temperature was 25+/-2°C, the relative humidity was 70 +/- 15%, the photoperiod was 16h light/8h dark with 200-3000 lux. There were short deviations to these conditions but this did not affect the performance and the quality of the test. The fecundity performance F was determined based on the following equation:
F = (eggs laid in unit 1 + eggs laid in unit 2) * (% hatching eggs unit 1).

The results of the assessments are summarized in the table 10.3.2.2-1 below **(Simulated values)**

Table 10.3.2.2-1: Effects of NUTREL to *Orius laevigatus* on natural substrate

Test product	Observed mortality (%)	Corrected mortality (%)	Fecundity performance (F) (%)
Control	N.A.	N.A.	N.A.
NUTREL 378 g/L (2.4 L/ha)	N.A.	N.A.	N.A.
Dimethoate	N.A.	N.A.	Not determined

Conclusion

No effect on the reproductive capacity was observed for this test rate.

Study Comments: IIIA 10.3.2.2/01	
Agreed endpoint/s: IIIA 10.3.2.2	

Report:	No data available*
Title:	Dose-response study to assess side-effects of NUTREL on adults of the parasitic wasp *Aphidius rhopalosiphi* (Destefani-Perez) (Hym.; Aphidiidae) on plants in the laboratory
Document No:	Data gap
Guidelines:	Mead-Briggs & Longley (1997)
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Summary

In theory, the study encompassed 7 treatment groups (3 dose rates of the test item NUTREL, control and dimethoate reference item) with 5 replicates each containing 5 female adult wasps. Only two replicates each containing 5 female adult wasps were used for the toxic standard. The adult wasps were exposed to dry residues on treated barley seedlings for 48 hours. At the end of this period, the surviving females were removed for fecundity assessments. The parasitized aphids within the fecundity arenas were left to develop in situ and the number of aphid mummies that developed was recorded 12 days later.

The temperature was 20+/-3°C, the relative humidity was 60-90%, the photoperiod was 16h light/8h dark. There were short deviations to these conditions (10-25°C and 30-100% RH for less than 2 hours) but this did not affect the performance and the quality of the test. The percentage mortality of wasps was calculated as well as the mean number of mummies produced per wasp.

The results of the assessments are summarized in the table 10.3.2.2-2 below **(Simulated values)**

Table 10.3.2.2-2: Effects of NUTREL to *Aphidius rhopalosiphi* on natural substrate

Test product	Observed mortality (%)	Corrected mortality (%)	Number of mummies per female wasp
Control	N.A.	-	N.A.
NUTREL (500 g a.i./ha)	N.A.	N.A.	N.A.
NUTREL (600 g a.i./ha)	N.A.	N.A.	N.A.
NUTREL (1000 g a.i./ha)	N.A.	N.A.	N.A.
Dimethoate	N.A.	N.A.	N.A.

Conclusion

No effect on the reproductive capacity was observed for any test rate.

Study Comments: IIIA 10.3.2.2/02	
Agreed endpoint/s: IIIA 10.3.2.2	

Report:	No data available*
Title:	Side-effects of NUTREL on larvae of the lacewing *Chrysoperla carnea* L. (Nevroptera; Chrysopidae) on plants in the laboratory
Document No:	Data gap
Guidelines:	Bigler and Waldburger for exposure on group of whole plants (1988) and Vogt et al. (2000)
GLP	Yes

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Summary

In theory, the study encompassed 4 treatment groups (2 dose rates of the test item NUTREL , control and dimethoate reference item) with 4 replicates each containing 15 lacewing larvae. Only one replicate each containing 15 lacewing larvae was used for the toxic standard. The larvae were exposed to dry residues on broad beans for 10 days at 15-25°C and were left undisturbed during this period. At the end of the exposure phase, units were dismantled and lacewing larvae and pupae were harvested. Larvae were thus individually confined in plastic petri dishes and fed until pupation. All adults that emerged were released into fertility units. Females were assessed for fertility performance by counting the eggs obtained from two 24h egglaying periods. Viability of the eggs laid was assessed and number of viable eggs produced per female per day was used to calculate the reproductive ratio (R).

The temperature was 15-25°C, the relative humidity was 50-90%, the photoperiod was 16h light/8h dark with at least 3000 lux. There were short deviations to these conditions (10-30°C and 30-100% RH for less than 2 hours) but this did not affect the performance and the quality of the test. The percentage mortality was calculated as well as the hatching rate and the mean number of viable eggs produced per female.

The results of the assessments are summarized in the table 10.3.2.2-3 below **(Simulated values)**

Table 10.3.2.2-3: Effects of NUTREL to *Chrysoperla carnea* on natural substrate

Test product	Observed pre-imaginal mortality (%)	Corrected mortality (%)	Number of viable eggs per female (% compared to control)
Control	N.A.	-	N.A.
NUTREL (500 g a.i./ha)	N.A.	N.A.	N.A.
NUTREL (1000 g a.i./ha)	N.A.	N.A.	N.A.
Dimethoate	N.A.	N.A.	N.A.

Conclusion

No effect on the reproductive capacity was observed for any test rate.

Study Comments: IIIA 10.3.2.2/03	
Agreed endpoint/s: IIIA 10.3.2.2	

CP 10.3.2.3 Semi-field tests

No extended lab studies were conducted as risk was shown to be acceptable based on tests with artificial substrates.

CP 10.3.2.4 Field studies with non target arthropods

No extended lab studies were conducted as risk was shown to be acceptable based on tests with artificial substrates.

CP 10.3.3. Other routes of exposure for non-target arthropods

No data available.

CP 10.4 Effects on Non-Target Soil Meso- and Macrofauna

CP 10.4.1 Earthworms
Risk assessment for earthworms
CP 10.4.1.1 Earthworms – sub-lethal effects
CP 10.4.1.2 Earthworms – field studies

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)
Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)
CP 10.4.2.1 Species level testing
CP 10.4.2.2 Higher tier testing

CP 10.4 Effects on Non-Target Soil Meso- and Macrofauna
Effects on Earthworms and Other Soil Non-target Macro-organisms

EU endpoints: Effects on Earthworms and Other Soil Non-target Macro-organisms
Ecotoxicological endpoints for earthworms and other soil non target macro-organisms

Active substance	EU agreed endpoints (EFSA Journal 2012; 10(2):2545)	
Acute toxicity to earthworms		
Hydrolysed proteins	($LC_{50\ corr}$) No data available*	
Metabolite	Not required	
Preparation	Not required	
Chronic toxicity to earthworms		
Hydrolysed proteins	Not required	
Metabolite	Not required	
Preparation	Not required	
Other soil macro-organisms		
Not required		

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Summary

Effects on earthworms and other soil non-target macro-organisms of NUTREL were not evaluated as part of the EU review of Hydrolysed proteins. Therefore all relevant data and assessments are provided here and are considered adequate.

Table 10.4-1 Toxicity/exposure ratios for earthworms

Test substance	Use pattern	Species	Test type	Endpoint (mg a.i. /kg soil)	PEC (mg a.i. /kg soil)	TER	TER risk assessment trigger
Hydrolysed proteins	Air-assisted spraying	*Earthworm*	Acute	$LC_{50\ corr}$ N.A.	N.A.	N.A.	10

The TER value indicates that the use of NUTREL poses low risk to macro-organisms when applied according to the proposed use rates. Since Hydrolysed proteins are biodegradable with a DT_{90} in soil of few days (less than 100 days) and since only application is sought, neither earthworm chronic assessment nor studies on other macro-organisms are necessary.

As agreed during the EU review, no study with NUTREL was performed and found necessary since the acute TER based on the active substance is well above the trigger value (more than 100 fold). There are also no components in NUTREL that may enhance the toxicity of the active substance (see composition in the confidential Part C).

Toxicity

No acute macro-organisms toxicity study has been carried out with NUTREL since the risk was found to be very low based on the active substance data. No chronic earthworm toxicity study or studies on other macro-organisms were necessary since the DT_{90} for Hydrolysed proteins in soil is much less than 100 days and since only application is sought. Every detail of the acute study with Hydrolysed proteins is given in the EU DAR. The acute earthworm toxicity endpoint is summarised in Table 10.4-2.

Table 10.4-2: Summary of earthworm toxicity endpoints for Hydrolysed proteins

Parent compound	Test substance	Species	Endpoint	Value (mg/kg soil)	Reference
Acute toxicity studies					
Hydrolysed proteins	Hydrolysed proteins	Earthworms	LC_{50}	$LC_{50\ corr}$ * (N.A.)	***DAR (2008)***

* Based on standard assumptions of soil bulk density 1.5 g/cm^3 and incorporation depth of 5 cm.

Exposure

The exposure to soil organisms was estimated by calculating the maximum instantaneous predicted environmental concentrations in soil (PEC_S) (see Part B, Section 5).

The PEC_S after a single application was calculated using the following equation:

$$PEC_S(mg/kg) = \frac{Application\ \text{rate (g/ha)} \times (1\text{-}F)}{100 \times \text{Soil depth (cm)} \times \text{Soil dry bulk density (g/cm}^3\text{)}}$$

Where F is the fraction intercepted by the crop. NUTREL will be applied to the crops at BBCH 71-79, and therefore soil residues will be reduced by crop interception by 80%. The soil depth was assumed to be 5 cm and the soil dry bulk density was assumed to be 1.5 g/cm^3.

The resulting initial PEC_S value is presented in Table 10.4-3.

Table 10.4-3: Initial soil PEC value for Hydrolysed proteins

Parent compound	Substance	Maximum PEC_S (mg/kg)
Hydrolysed proteins	Hydrolysed proteins	N.A.

CP 10.4.1 Earthworms
Risk assessment for earthworms

▸Toxicity exposure ratios, TER_A and TER_{LT}

No study were submitted.

Acute risk

In theory, the potential acute risk of Hydrolysed proteins to earthworms following the use of NUTREL was assessed by comparing the maximum instantaneous PEC_S with the 14-day LC_{50} value to generate acute TER values. The log P_{OW} value of Hydrolysed proteins is higher than 2, and hence the LC_{50} must be reduced by a factor of 2 in order to account for the relatively high organic matter content of the artificial test soil compared to agricultural soils. The TER_A was calculated as follows:

$$TER_A = \frac{LC_{50}\ (mg/kg)}{PEC_S\ (mg/kg)}$$

The resulting TER_A value is shown in Table 10.4.1.

Table 10.4-1: Acute TER values for earthworms

Parent compound	Test substance	LC_{50} (mg/kg)	Maximum instantaneous PEC_S (mg/kg)	TER_A
Hydrolysed proteins	Hydrolysed proteins	$LC_{50\ corr}$ (N.A.)	N.A.	N.A.

The acute TER value is much higher than the Annex VI acute trigger value of 10, indicating that NUTREL poses low acute risk to earthworms when applied according to the proposed use rates. Because of the low soil DT_{90} for Hydrolysed proteins in soil (3-4 days), this conclusion applies to all soil macro-organisms.

Long-term risk

The potential long-term risk of Hydrolysed proteins to macro-organisms following the application of NUTREL was not calculated. However it was deemed to be very low since the DT_{90} for Hydrolysed proteins in soil ranges from 3-4 days and since only two to four applications of NUTREL per year is sought. No long term exposure of macro-organisms to Hydrolysed proteins is expected and the risk is deemed very low.

Comments: IIIA 10.4.1/01	
Agreed endpoint/s: IIIA 10.4.1	

▸Acute toxicity

As agreed during the EU review, no study with the preparation NUTREL was performed and found necessary since the acute TER based on the active substance is well above the trigger value (more than 100 fold).
There are also no components in NUTREL that may enhance the toxicity of the active substance (see composition in the confidential Part C).

Comments: IIIA 10.4.1/02	
Agreed endpoint/s: IIIA 10.4.1	

CP 10.4.1.1 Eathworms - Sublethal effects

No studies on sublethal effects were conducted with the formulation since the DT_{90} for

Hydrolysed proteins in soil ranges from 3 to 4 days and since only two to four applications of NUTREL per year is sought.

CP 10.4.1.2 Eathworms - Field studies

No field tests were conducted as assessment of the lab studies showed exposure to be acceptable.

CP 10.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms) Risk assessment for other non-target soil meso- and macrofauna (other than earthworms

No studies to assess the effects of the formulation to other soil macro-organisms were conducted since the DT_{90} for Hydrolysed proteins in soil ranges from 3 to 4 days and since two to four applications of NUTREL per year is sought.

CP 10.4.2.1 Species level testing Residue content of earthworms

No study were submitted.
However, in theory the residues in earthworms are calculated by multiplying the PEC soil by the bioconcentration factor for earthworms.

1. PEC soil
Calculations are made by using the equation provided in Guidance Document 7193/VI and can be found in Section 5, Annex point IIIA 9.4.1. The resulting PEC values are presented in the table below.

Table 10.4.2-1. Initial, actual and TWA PEC_s (mg/kg) of Hydrolysed proteins following the application of NUTREL in all crops (1000 g a.s./ha)

$PEC_{(s)}$		Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial		N.A.	-	-	-
Short term	24h	N.A	N.A	-	-
	2d	N.A	N.A	-	-
	4d	N.A	N.A	-	-
Long term	7d	N.A	N.A	-	-
	14d	N.A	N.A	-	-
	21d	N.A	N.A	-	-
	28d	N.A	N.A	-	-
	50d	N.A	N.A	-	-
	100d	N.A	N.A	-	-

2. BCF for earthworms

The BCF for earthworms is calculated as:

$$BCF = \frac{(0{,}84 + 0{,}01 * P_{ow})}{f_{oc} * K_{oc}}$$

where K_{oc} = 896 L/kg
f_{oc} = 0.02 (standard value for the organic carbon content of soil)
P_{ow} = 144.54 (calculated from log P_{ow} = 2.16)

The resulting BCF of Hydrolysed proteins for earthworms determines kg of the fresh weight of earthworms per kg soil dry weight.

3. Calculation of the Hydrolysed proteins residue in earthworms

Residue levels in earthworms were calculated as follows:

$$PEC_{worm} = PEC_{soil} * BCF_{worm} = 0.0068 \text{ mg a.i./kg}$$

CP 10.4.2.2 Higher tier testing
Effects on organic matter breakdown

No studies to assess the effects of the formulation on the organic matter breakdown were conducted since the DT_{90} for Hydrolysed proteins in soil ranges from 3 to 4 days and since two to four applications of NUTREL per year is sought.

CP 10.5 Effects on Soil Nitrogen Transformation

Risk assessment for Soil Nitrogen Transformation
Effects on Soil Microbial Activity

No study were submitted.

EU Endpoint: Effects on Soil Microbial Activity

Ecotoxicological endpoints for soil micro-organisms

Active substance	Test design	EU agreed endpoints (EFSA Journal 2012; 10(2):2545)	Remark
Hydrolysed proteins	N	Nitrogen mineralization	No data available*
	C	Carbon mineralization	No data available*

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

Summary

Effects on soil microbial activity of NUTREL were not evaluated as part of the EU review of Hydrolysed proteins. The effects were however evaluated for the active ingredient during the EU review. These results can be extrapolated to NUTREL because it is a soluble liquid formulation which mainly contains active ingredient and water (cfr also Part C of the dRR containing the confidential information).

In our opinion there is no additional data needed concerning the risk to micro-organisms of hydrolysed proteins (DAR 2008).

Therefore, the application of NUTREL poses low risk to micro-organisms.

CP 10.6 Effects on Terrestrial Non-Target Higher Plants

Risk assessment for Terrestrial Non-Target Higher Plants

CP 10.6.1 Summary of screening data

CP 10.6.2 Testing on non-target plants

CP 10.6.3 Extended laboratory studies on non-target plants

CP 10.6.4 Semi-field and field tests on non-target plants

CP 10.6 Effects on Terrestrial Non-Target Higher Plants

Agreed EU End-points used in the Evaluation (EFSA Journal 2012; 10(2):2545)

CP 10.6.1 Summary of screening data

Not required

Laboratory dose response tests

Test substance	Most sensitive species	ER50 (g/ha)[2] Vegetative vigour	ER50 (g/ha)[2] Emergence	Exposure[1] (g/ha)[2]	TER	Trigger
Hydrolysed proteins	No data available*	/	/	/	/	5

[1]Formulation endpoint is espressed in terms of g a.s. 7 ha

[2]Off-crop soil PEC, based on a maximum application rate of 1000 g a.s./ha (to all crops) and a default drift value at 3 m of field crops and assuming a soil depth of 5 cm and density of 1.5 g/cm^3

**Once components are known, and the environmental exposure can be finalised a risk assessment, should be provided to address the risk of Hydrolysed proteins to no-target organisms, whenever the exposure to environment will be greater than the natural background level.*

End-points used in Evaluation (not necessarily previously agreed)

Active substance	Test design[1]	Endpoints used in the assessment
Hydrolysed proteins	Seedling emergence	EC_{50} (mg a.i./kg soil)* No data available
	Vegetative vigour	EC_{50} (g a.i./ha)* No data available

*Since Annex I inclusion new studies on the formulation have been performed and as a result there are new end-points which are used in the risk assessment

Summary

NUTREL was not a representative formulation in the EU review of Hydrolysed proteins. Therefore all relevant data and assessments are provided here and are considered adequate. In theory, the risk assessment is based on studies performed with the formulation NUTREL for the vegetative vigour and the seedling emergence.

Table 10.6-1 Effects on non-target plants for a default 3 meter no spray bufferzone

Test substance	Test method	Species	ER_{50}	$PER_{off\text{-}field}$	TER	Trigger value
NUTREL	*Vegetative vigour*	*Raphanus sativus*	(g a.i./ha) N.A.	(g a.i./ha) N.A.	N.A.	5
		Lepidium sativum	(g a.i./ha) N.A.	(g a.i./ha) N.A.	N.A.	5
		Solanum lycopersicum	(g a.i./ha) N.A.	(g a.i./ha) N.A.	N.A.	5
		Daucus carrota	(g a.i./ha) N.A.	(g a.i./ha) N.A.	N.A.	5
		Avena sativa	(g a.i./ha) N.A.	(g a.i./ha) N.A.	N.A.	5
		Allium cepa	(g a.i./ha) N.A.	(g a.i./ha) N.A.	N.A.	5
	Seedling emergence	*Beta vulgaris*	(mg a.i./kg soil) N.A.	(mg a.i./kg soil) N.A.	N.A.	5
		Sinapis alba	(mg a.i./kg soil) N.A.	(mg a.i./kg soil) N.A.	N.A.	5
		Lactuca sativa	(mg a.i./kg soil) N.A.	(mg a.i./kg soil) N.A.	N.A.	5
		Vigna radiata	(mg a.i./kg soil) N.A.	(mg a.i./kg soil) N.A.	N.A.	5
		Avena sativa	(mg a.i./kg soil) N.A.	(mg a.i./kg soil) N.A.	N.A.	5
		Zea mays	(mg a.i./kg soil) N.A.	(mg a.i./kg soil) N.A.	N.A.	5

Value in bold is under the trigger value

CP 10.6.2 Testing on non-target plants
Terrestrial plants

Toxicity
The potential effects of NUTREL on seedling emergence and vegetative vigour of 3 non-target terrestrial plants has been tested; there were no observable effects on seedling emergence, or vegetative vigour at rates up to the highest tested concentration of 10 mg/kg dry soil. Further details of the study are given in the summary at Point 10.8.1.3 below.

According to the Terrestrial Guidance Document[8], the risk to non-target plants should be considered acceptable if the TER is higher than 5. The calculation shows that TER is higher than 5 for an application of 1000 g a.i./ha in all crops with a default 3 meter no spray buffer zone. Hence, no risk mitigation measure is necessary for the use of NUTREL in all crops.

Risk assessment
NUTREL is a insect attractant and is therefore not expected to have any significant herbicidal activity. A profiling study of the effects on pre- and post-emergence non-target higher plants was conducted and showed no effects on any of the 3 species tested at rates up to the highest tested concentration of 10 mg/kg dry soil.

CP 10.6.2.1 Seed germination

This is not an EC data requirement / not required by Directive 91/414/EEC.

CP 10.6.2.2 Vegetative vigour

No vegetative vigour study performed on NUTREL.

Study Comments: IIIA 10.6.2.2/01	
Agreed endpoint/s: IIIA 10.6.2.2	

[8] Anonymous (2002b). Guidance Document on terrestrial ecotoxicology under council directive 91/414/EEC. SANCO/10329/2002. 17 October 2002.

CP 10.6.3 Extended laboratory studies on non-target plants Seedling emergence

No field study performed on NUTREL.

Study Comments: IIIA 10.6.2.3/01	
Agreed endpoint/s: IIIA 10.6.2.3	

CP 10.6.4 Semi-field and field tests on non-target plants

No field and semifield testing conducted as risk assessment was made based on results from laboratory tests.

Study Comments: IIIA 10.6.4/01	
Agreed endpoint/s: IIIA 10.6.4	

CP 10.6.4.1 Effects on non-target aquatic plants

Tests on aquatic plants are not required as NUTREL is not an herbicide.
Please refer to assessment made under IIIA 10.2

CP 10.6.4.2 Lemna growth test

See point above.

CP 10.6.4.3 Field tests

Not necessary since it was concluded that NUTREL poses low risk to aquatic plants based on the tier 1 assessment. See point 10.6.2.5.

CP 10.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

Risk assessment for Other Terrestrial Organisms (Flora and Fauna)

CP 10.7 Other Non-Target Species (Flora and Fauna)

Tests on other non-target species are not required.

CP 10.7.1 Available preliminary data on other non-target species (flora and fauna)

No data on other non-target species is required.

Study Comments: IIIA 10.7.1/01	
Agreed endpoint/s: IIIA 10.7.1	

CP 10.7.2 Critical assessment of relevance of preliminary test data

None relevant.

CP 10.8 Monitoring Data

CP 10.8.1 Non-target species at risk and extent of potential exposure

There are no additional European requirements for formulated products.

CP 10.8.2 Short and long term risks for non-target species, populations, communities and processes

There are no additional European requirements for formulated products.

CP 10.8.3 Risk of fish kills and fatalities in large vertebrates or terrestrial predators

There are no additional European requirements for formulated products.

CP 10.8.4 Precautions necessary to avoid/minimise environmental contamination and to protect non-target species

Use of NUTREL at the proposed label rates and according to good agricultural practice poses low risk to all non-target species without any need of mitigation measures.

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