Hydrolysed proteins

DOCUMENT M-CA, Section 8

ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE

Version history[1]

A) BIO

Date	Data points containing amendments or additions and brief description	Document identifier and version number
2005-26-06	Initial Document M version, submitted for application of approval of the active substance.	M-Hydr.Protein-AnnexII
2018-01-09	Two acute toxicity studies on Daphnia sp and on Brachydanio rerio (fish) are presented. Both studies were performed in a GLP complying laboratory and tested the active substance called "BIOCEBO" (35 % w/w hydrolysed proteins). A bibliographical analysis of the effects of hydrolysed proteins on aquatic organisms. These new data were presented to comply with Regulation 571/2012, amending Regulation (EU) No 540/2011, which establishes that for the hydrolysed proteins some additional information is required regarding the risk for aquatic organisms.	DOCUMENT M-CA, Section 8 CA 8.2.4.1 Acute toxicity to Daphnia magna (Former Section 3A of the Document M, Annex II). CA 8.1.1 Further testing on aquatic organisms

[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

B) PHY

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

C) SIC

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

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CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE (BIO)

Introduction

Hydrolysed proteins are natural compounds of degradation from the hydrolysis of living organism's tissues, that can have vegetable or animal origin. Proteins are the most abundant organic molecules in cells. They constitute the 50% of the dry weight of cells, or even more. They can be found in every single cell, since they are fundamental in all aspects of the cell structure and function (Lehninger, 1983).
The Animal and Vegetable cells are formed mainly by proteins, which constitute more than the half of the dry weight of the cell. Proteins determine the shape and structure of the cell and also function as an instrument of molecular recognition and of catalysis (Alberts, 1986).

Proteins have many different biological functions. The widest group of proteins are the enzymes whose function is about catalysing the biochemical processes that take place in the living organisms. Moreover, there are proteins of reservation of amino acids such as plant nutrients; transport proteins of specific molecules; proteins that work as essential elements of the motile and contractile systems; protective proteins that are present in the blood of the vertebrates such as antibodies; proteins that function as hormones and, finally, structural proteins (Lehninger, 1983).

The proteins that are found in food and eaten by human beings and mammals are normally degraded metabolically by means of enzymatic processes to give rise to more simple metabolites (parotids and amino acids) that are used by the live cells for the biosynthesis of new specific proteins. Therefore, they do not cause any danger to human beings and mammals in general. As it has been explained before, proteins appear in all biochemical processes that take place in every live cell being, this way, essential compounds for human life.

Furthermore, hydrolysed proteins are authorized by the EU in order to be used as attractant in the elaboration of baits in combination with appropriate insecticides of the Organic Farming (Regulation EC 889/2008 annex 2). This shows the innocuousness of these compounds, since the practice of this kind of agriculture is very demanding with the use of products that can be harmful to human beings.

The active ingredient Hydrolysed proteins means polypeptides, peptides and amino acids and mixtures thereof obtained by hydrolysis of animal by-products. The main health hazard of concern with hydrolysed proteins derived from animal by-products is the risk of BSE/TSE (Bovine spongiform encephalopathy/transmissible spongiform encephalopathy) contamination or of microbial human pathogens contamination. In order to exclude the risk of contamination of the raw animal material, the manufacturing process and the plant facilities are in accordance to the requirements of the Regulation 1069/2009, laying down health rules concerning animal by-products not intended for human consumption.

The Draft Assessment Report of Greece stated in 2008 that RMS accepted the argumentation that the active substance hydrolysed proteins derived from hydrolysis of animal tissues do not have any significant toxicity potential. The overall conclusions included there, in point 3, states: *"there are clear indications that it may be expected that hydrolysed proteins does not have any harmful effects on human or animal health or on groundwater or any unacceptable influence on the environment, as set out in Annex VI of regulation (EC) 2229/2004 as last amended by Regulation (EC) 1095/2007"*.
Regulation 571/2012, amending Regulation (EU) No 540/2011 as regards to the conditions of approval of the active substances aluminum silicate, hydrolysed proteins and 1,4-diaminobutane (putrescine), establishes that for the hydrolyzed proteins some additional information is required regarding the risk for aquatic organisms.

In this mentioned Regulation, it is also stated that applicants shall submit the information requested to the European Commission through the Rapporteur European Member State (Greece in this case), by 1st of November 2013. BIOIBERICA S.A., as one of the applicants for the inclusion of Hydrolyzed proteins in Annex I of the derogated Directive 91/414, concerning the placing of plant protection products on the market, has accordingly prepared two acute toxicity studies on *Daphnia sp* and on *Brachydanio rerio* (fish). Both studies were performed in a GLP complying laboratory (Eurofins Biolab S.r.l., located in Italy) and tested the active substance called "BIOCEBO" (35 % w/w hydrolyzed proteins). Results showed that BIOCEBO (which in this case is not the PPP), supposes no risk for aquatic organisms such as *Daphnia* or *Brachydanio rerio* in acute toxicity tests such as acute Immobilisation Test and Limit Test (equilibrium loss, irregular swimming, difficulties in respiratory functions and variation of pigmentation), respectively.

Moreover, BIOIBERICA, S.A. together with the other two applicants, requested to IRTA (Institute for Food and Agricultural Research and Technology) a bibliographical analysis of the effects of hydrolysed proteins on aquatic organisms. The results of this analysis concluded that there is no evidence for any effects of hydrolysed proteins on aquatic ecosystems in general and on aquatic organisms in particular.

The next pages present the results of these three studies. The complete reports are located in the J-Hydr.protein directory.

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

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CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

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CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

STUDY NUMBER 1. Acute toxicity on aquatic organisms (Daphnia sp): EC50 TEST ON "BIOCEBO"

Introduction:

The aim of this study was to determine the ecotoxicological effects "BIOCEBO" (hydrolyzed proteins ≥35 % w/w), on biotic systems putting as a model the aquatic organism *Daphnia magna* as a test system to perform the EC_{50} test.

This acute immobilization test was evalutated according to the OECD guideline N.202.

Report:	S-2013-01827 AM
Title:	Acute toxicity on aquatic organisms (Daphnia sp): EC50 TEST ON "BIOCEBO"
Test facilities:	Eurofins Biolab S.r.l. of Vimodrome (MI)-via B. Buozzi n.2 (Italy)
Guidelines:	OECD Guidelines for the testing of Chemicals/Section 2: Effects on Biotic Systems Test Nº. 202: Daphnia sp. Acute Immobilisation Test 2004
GLP	Yes

Material and Methods:

The organisms were exposed to 5 different solutions of BIOCEBO for a total period of 48 hours, the number of immobilized organisms and/or possible abnormal behaviors were observed.

120 Daphnia were used, 100 of them treated with different concentrations and 20 as a control (no Biocebo addition). 4 replications for every condition were prepared, afterwards Daphnia was added to the vessels of the assay sample.

Dissolved oxygen, pH and temperature of the assay were measure at the beginning and at the end of the test.

Validity criteria:

- The immobilization of control animals must not be higher than 10% at the end of the test.
- pH values must not change for more than 1.5 units
- The concentration of dissolved oxygen in the vessels must not go below 2 mg/l.

Results:

Table 1: Number of Daphnia immobilized at 24 and 48 hours in treated and control conditions

Group	Replication Nº	Concentration of BIOCEBO administered	Nº Daphnia individuals immobilized at 24h	Nº Daphnia individuals immobilized at 48h
Treated	1	1.00 g/l	1/5	2/5
	2		0/5	1/5
	3		1/5	1/5
	4		0/5	0/5
Treated	1	0.50 g/l	0/5	0/5
	2		0/5	1/5
	3		0/5	1/5
	4		0/5	1/5
Treated	1	0.25 mg/l	0/5	0/5
	2		0/5	0/5
	3		0/5	0/5
	4		0/5	0/5
Treated	1	0.125 mg/l	0/5	0/5
	2		0/5	0/5
	3		0/5	0/5
	4		0/5	0/5
Treated	1	0.063 mg/l	0/5	0/5
	2		0/5	0/5
	3		0/5	0/5
	4		0/5	0/5
Control	1	0.00 mg/l	0/5	0/5
	2		0/5	0/5
	3		0/5	0/5
	4		0/5	0/5

n=5 for each condition

Table 2: Concentration of dissolved oxygen (mg/l) in treated (1 g/l) and control at the beginning and at the end of the test (48 hours).

	pH		**Dissolved oxygen**	
	Beginning of the test	End of test	Beginning of the test	End of test
Control				
Replication nº 1	8.18	8.72	6.61	5.65
Replication nº 2	8.20	8.74	6.64	5.69
Replication nº 3	8.15	8.70	6.58	5.61
Replication nº 4	8.17	8.71	6.67	5.67
Concentration 1.00 g/l				
Replication nº 1	7.40	8.14	7.21	3.75
Replication nº 2	7.43	8.12	7.17	3.78
Replication nº 3	7.39	8.16	7.22	3.73
Replication nº 4	7.41	8.15	7.25	3.77

Findings:

- pH values were not significantly different between the two treatment conditions.
- Dissolved oxygen was lower at the end of the test with the highest concentration of BIOCEBO but validity criteria was satisfied.
- Temperature did not change during the test.
- The obtained results showed that the *Daphnia magna* EC_{50} after 48 hours at the concentration of 100 mg/l of the test item "BIOCEBO" is higher than 1.00 g/l.
- All the test parameters satisfied the validity criteria.

Conclusion/endpoint:

Results showed that BIOCEBO supposes no risk for aquatic organisms such as *Daphnia*.

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

STUDY NUMBER 2. *Brachydanio rerio*, acute toxicity test-limit test: on "BIOCEBO".

Introduction:

The aim of this study was to determine the ecotoxicological effects "BIOCEBO" (hydrolyzed proteins ≥35 % w/w), on biotic systems putting as a model the aquatic organism *Brachydanio rerio* as a test system to perform a limit test.

This acute immobilization test was evaluated according to the OECD guideline N.203.

Report:	S-2013-01828 AM
Title:	Brachydanio rerio, ACUTE TOXICITY TEST-LIMIT TEST: ON "BIOCEBO".
Test facilities:	Eurofins Biolab S.r.l. of Vimodrome (MI)-via B. Buozzi n.2 (Italy)
Guidelines:	OECD Guidelines for the testing of Chemicals/Section 2: Effects on Biotic Systems Test Nº. 203: Fish, Acute Toxicity Test 1992
GLP	Yes

Material and Methods:

The organisms were exposed to 100 mg/l of BIOCEBO or control solutions for a total period of 96 hours. All visible abnormalities such as equilibrium loss, irregular swimming, difficulties in respiratory functions and variation of pigmentation were measured.

14 fishes were used, 7 of them treated with the BIOCEBO with a concentration of 100 mg/l and other 7 were used as control, in the same assay conditions than without adding BIOCEBO.

At the different observations intervals pH, dissolved oxygen and assay water temperature were measured.

Validity criteria:

- The mortality in the control animals should not exceed one fish at the end of the test.
- The dissolved oxygen concentration must have been at least 60 percent of the air saturation value throughout the test.

Results:

Table 1: Number of dead fishes in control and treated during the assay

Group	Nº of animals	Concentration of BIOCEBO administered	24 hours	48 hours	72 hours	96 hours
Treated	7	100 mg/l	0	0	0	0
Control	7	0.00 mg/l	0	0	0	0

Table 2: Concentration of dissolved oxygen (mg/l) in treated (1 g/l) and control at the beginning and at the end of the test (48 hours).

Group	0 hours	24 hours	48 hours	72 hours	96 hours
pH values					
Control	7.90	8.20	8.30	8.18	8.11
Treated (100 mg/L)	7.75	8.08	8.14	8.10	8.10
% of saturation of dissolved oxygen					
Control	70.0	67.4	67.1	66.7	66.5
Treated (100 mg/L)	71.8	65.7	64.5	63.7	62.8

Findings:

- pH values remained within the required limits (6,0-8,5).
- The percentage of saturation has remained for the whole length of the assay above 60% both in control and in treated conditions.
- Temperature remained in the interval required for the species.
- The obtained results showed that no case of mortality was declared in treated animals and in control ones. No toxic symptom was detected neither.
- All the test parameters satisfied the validity criteria.

Conclusion/endpoint:

The obtained results, in compliance with assay validity criteria, showed that no dead fishes at 100 mg/l of BIOCEBO after 96 hours were observed.
Results showed that BIOCEBO supposes no risk for aquatic organisms such as Brachydanio.

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

CA 8.2.5.4 Sediment dwelling organisms

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

CA 8.2.6.2 Effects on growth of an additional algal species

CA 8.2.7 Effects on aquatic macrophytes

CA 8.2.8 Further testing on aquatic organisms

STUDY NUMBER 3. Bibliographical analysis of the effects of hydrolysed proteins on aquatic organisms

Introduction:

The objective and scope of this study was to make a complete and systematic technical and scientific bibliographical review of the effects that the use of protein hydrolysate baits may have on the aquatic organisms.

Report:	-
Title:	Bibliographical analysis of the effects of hydrolysed proteins on aquatic organisms.
Test facilities:	IRTA: Institute for Food and Agricultural Research and Technology
Guidelines:	Not applicable
GLP	Not applicable

Material and Methods:

To do so, the following database and open access search engine and repositories have been consulted: Web of Knowledge, Google Scholar, AGRIS, Aquatic Commons, OceanDocs, OAister WorldCat and OpenDOAR.

The search strategy consisted on different key words used such as “hydrolysed protein” and “aquatic organisms” and the Boolean operators applied to combine them.

Results:

- Through the different searches, several records were found but none of them was relevant to the subject.

Conclusion/endpoint:

The obtained results of the systematical bibliographical search showed no effects of any kind of the use of hydrolysed protein baits on aquatic organisms.

Therefore, there is no evidence for any adverse effects of hydrolysed proteins on aquatic ecosystems in general and on aquatic organisms in particular.

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

CA 8.3.1.1.2 Acute contact toxicity

CA 8.3.1.2 Chronic toxicity to bees

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

CA 8.3.1.4 Sub-lethal effects

CA 8.3.2 Effects on non-target arthropods other than bees

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

CA 8.3.2.2 Effects on *Typhlodromus pyri*

CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna

CA 8.4.1 Earthworms – sub-lethal effects

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

CA 8.5 Effects on Nitrogen Transformation

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

CA 8.6.1 Summary of screening data

CA 8.6.2 Testing on non-target plants

CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

CA 8.8 Effects on Biological Methods for Sewage Treatment

CA 8.9 Monitoring Data

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE (PHY)

Introduction

PHYTOPHYL manufactures "Hydrolysed Protein" which is made of Beet molasses and Urea. Both of them are used very widely for many years and have not ever classified as dangerous substances.

Beet molasses are a natural by-product of the sugar industry, defined as the end product of sugar manufacture or refining from which no more sugar may be economically crystallized by conventional means.

Beet molasses mainly used for two purposes, Animal feed additive and Alcohol Production.

There is no evidence in bibliography that Beet molasses are for some reason toxic, irritant or ecologically unsafe.

PHYTOPHYL & FORESTRY COMISSION notified urea according to 91/414 and the substance is now approved under Reg. (EC) No 1107/2009. No toxicity studies were submitted but literature data about the toxicity of urea indicated limited toxicological potential.

During this first notification and inclusion Urea was not registered to ECHA but now has a full registration, the dossier is evaluated and there are 163 active registrants as a high volume chemical (production of 10.000 000 – 100.000.000 TONNES per year).

The annual application rate for urea, or hydrolysed protein in case of ENTOMELA 50SL for 6 applications per year according to the table of intended uses (CP 3.3) is:

Application rate per year for each active substance and total nitrogen content **(6 applications/year)**	
Hydrolysed protein	1.8kg – 2.08 kg/ha
Urea	0.576 kg – 0.648kg kg/ha
Total nitrogen content	0,288-0.333kg/ha

These rates are very low if we compare them to the annual application rates for urea as fertilizer which are reported to the ECHA site and are 60kg, 120kg, 180kg N/ha.

We can see that the use of Nitrogen fertilizers emits 180-540 times more nitrogen to the environment than the use of ENTOMELA 50SL for bait sprays and the quantities of urea and beet molasses that liberated to the environment are very low in comparison to the use of similar compounds as fertilizer or other uses, or even the quantities of them in wastewater of human origin.

An additional provision of the approval of hydrolysed /2012 (amending Implementing Regulation EU No 540/2011) was that the applicant submits to the Commission confirmatory information as regards: The risk to aquatic organisms.

PHYTOPHYL with the other two applicants for the inclusion of Hydrolysed protein prepared a literature review in a private Spanish Laboratory IRTA. On concluding remarks of this study refers that "There is therefore no evidence for any adverse effects of hydrolysed proteins on aquatic ecosystems in general and on aquatic organisms in particular".

PHYTOPHYL submit a DRR for ENTOMELA 50SL on 2015 according to reg. 1107/2009 and below are the Overall comments of zRMS on Ecotoxicological studies section:

zRMS overall comments:

Hydrolysed proteins (beet molasses hydrolysate)

No toxicity studies on non-target organisms were submitted during the EU peer review of the active substance hydrolysed proteins. However, according to the Draft Assessment Report for hydrolysed proteins (Volume 3, Annex B-9: Ecotoxicology, April 2008) due to the nature of the active substance and the characteristics regarding its fate and behaviour in the environment (biodegradable, non-persistent, non bioaccumulative), the use of hydrolysed proteins is considered of low danger for the terrestrial and aquatic wildlife and ecosystem in general. In fact, the available data indicate that it may be expected that hydrolysed proteins do not have any unacceptable influence on the environment.

However, two points regarding the environmental effects and risk assessment were raised during the EU peer review of the active substance hydrolysed proteins (EFSA Journal 2012; 10(2):2545): i) The necessity of toxicity studies on aquatic organisms directly related to the classification and labelling and ii) The requirement of environmental exposure assessments to soil, surface water, aquatic sediment and groundwater. Depending on the outcome of these assessments (whether the exposure to the environment arising from the representative uses is greater than the natural background level) risk assessments to non-target organisms (birds and mammals, aquatic organisms, bees, non-target arthropods, non-target soil organisms, non-target plants) may be required.

In order to address the former point, the three Notifiers for the inclusion of hydrolysed proteins in Annex I to Council Directive 91/414/EEC (BIOIBERICA S.A., PHYTOPHYL – N.G. STAVRAKIS, SICIT 2000 S.p.A.) have provided confirmatory data which are presented in the Addendum IV to DAR (Volume 3, Annex B-9: Ecotoxicology, September 2014). The EU evaluation of these confirmatory data is currently under progress. Based on the submitted data the designated RMS (Greece) has reached the following conclusion:

Although no specific testing toxicity data on either of the hydrolysed proteins notified (animal tissue hydrolysate, beet molasses urea hydrolysate, collagen protein hydrolysate) have been submitted, taking into account:

- *(i)* the lack of any information *or evidence in the scientific literature related to the aquatic toxicity potential of hydrolysed proteins,*
- *(ii) the indication of low hazard and risk associated with the use of hydrolysed proteins (e.g. beet molasses-urea hydrolysate) in insect attractants for bait spray applications compared to other nitrogen compounds (e.g. fertilizers) and*
- *(iii)the nature of the active substance and its characteristics regarding the fate and behaviour in the environment (biodegradable, non-persistent, non bioaccumulative),*

it can be concluded that the use of hydrolysed proteins is of low danger for the aquatic ecosystems in general and for the aquatic organisms in particular. In consequence, from the RMS's point of view,

hydrolysed proteins should not be assigned any classification for aquatic hazards and should be deemed as non-dangerous for the environment substances.

It should be also noted that the applicant (PHYTOPHYL – N.G. STAVRAKIS) has provided a "Genetically Modified Organisms Free" certificate to the zRMS which ascertains and confirms that the starting material for the preparation of hydrolyzed proteins (beet molasses) is not obtained from/does not contain genetically modified plants.

Urea

A considerable amount of information on the ecotoxicological potential of urea based on reviews by other organizations (US EPA, SIDS, IUCLID database) has been made available during the EU peer review of the active substance urea. The available information indicate limited toxicological potential and low ecotoxicity to non-target organisms. In addition, studies on the environmental fate and behaviour of urea clearly demonstrate that urea is highly soluble and biodegradable (both in soil and aquatic compartment), and it is not subject to accumulation within the ecosystem (log P_{OW} = -1.59 at 20-25°C). It has to be also noted that urea has been applied to growing arable crops and to pasture as one of the commonest nitrogen fertilisers for many decades, with few contra-indications. It is thus perhaps not surprising that assessments of the toxicity of the substance would not suggest it is hazardous by nature (DAR, Volume 3, Annex B-9: Ecotoxicology, April 2008).

Regarding aquatic environment, the available information was considered sufficient taking into account the nature of the active substance and that all of the toxicity values provided were consistent of low toxicity to aquatic organisms. In addition, biodegradation is expected to be the major fate process of urea in the aquatic ecosystem. Overall the proposed use of urea was considered to pose low risk to aquatic organisms (Addendum I to DAR, Annex B-9: Ecotoxicology, June 2011).

However, two points regarding the environmental effects and risk assessment were raised during the EU peer review of the active substance urea (EFSA Journal 2012;10(1):2523): i) The necessity of submission of the original ecotoxicological studies presented in the DAR and more specifically of the aquatic toxicity studies directly related to the classification and labelling of the active substance (the information available was limited to summaries of US EPA and OECD assessments and the IUCLID database), ii) The requirement of risk assessments to non-target organisms pending on the finalization of the environmental exposure assessments of urea and its transformation products (consideration of whether the exposure to the environment arising from the representative uses of the active substance is greater than the natural background level). This requirement is relevant for spray uses to conifer stumps and olive trees.

Taking into account the available information on the two active substances, the intended uses of ENT50 (soluble concentrate of urea with beet molasses) in spot bait spray applications is not expected to pose any unacceptable effects to non-target organisms (birds, aquatic organisms, wild mammals, bees, non-target arthropods, earthworms and other soil-macroorganisms, soil microorganisms and non-target plants) and no testing toxicity data are required.

There are not any new studies submitted by this dossier only an open literature review on MC-A Section 9 which will give more data about scientific knowledge during the last decade for hydrolysed proteins concerning, toxicity studies, relevant data and the potential risk for man and the environment.

All above mentioned justify that according to recent scientific knowledge there is no need for further eco-toxicological studies about the risk of Beet molasses-Urea hydrolysate.

In next paragraphs we are giving also ECHA presented data about eco-toxicological risk bout the active substance urea and IRTA literature study for aquatic organisms.

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates

No extra data for the ppp are presented only for urea below is the ECHA endpoint summary on terestrial toxicity:

"Urea is of inherently low toxicity and is rapidly assimilated into the nitrogen cycle by soil microorganisms; exposure is therefore limited.

The effects of long term use of urea fertiliser at 60, 120 and 180 kg N/ha/year was assessed on lumbricid earthworms in uncultivated turfgrass on loamy sand soil. The test sites were treated twice yearly for 20 years. Urea fertiliser reduced earthworm numbers and biomass and lowered pH. It was concluded that application of nitrogenous fertilisers for long periods may have a deleterious effect on earthworms in the absence of liming (Wei-Chum et al, 1990).

Low phytotoxicity is predicted for urea: the substance is widely used as a plant nutrient (N-source) in fertiliser, hence toxicity is unlikely.

The results of a study in soy bean plants confirm the low toxicity of urea.

Urea is of inherently low toxicity to microorganisms as it is utilised as a nutrient and nitrogen source. Testing of toxicity to soil microorganisms is scientifically unjustified.

The limited data available indicate that urea is of low toxicity to birds. A waiver is proposed for this endpoint on grounds of exposure."

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

CA 8.1.1.2 Short-term dietary toxicity to birds

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

CA 8.1.5 Endocrine disrupting properties

CA 8.2 Effects on Aquatic Organisms

Below is the summary and the remarks from IRTA study submitted on 2013 as confirmatory data for hydrolysed prpoteins:

Author: Institute for Food and Agricultural Research and Technology (IRTA)

Title: Bibliographical analysis of the effects of hydrolysed proteins on aquatic organisms

Executive summary

The objective and scope of this study was to make a complete and systematic technical and scientific bibliographical review of the effects that the use of protein hydrolysate baits may have on the aquatic organisms.
We present here the report of such a study, detailing the databases, search engines and open access repositories and catalogues consulted, as well as the search strategy performed (i.e. key words used and the Boolean operators applied to combine them).
The outcome of this review is that there is no evidence of any adverse effects of protein hydrolysate baits on aquatic organisms.

Concluding remarks

After a systematic bibliographical search as detailed above, no effects (of any kind) of the use of hydrolysed protein baits on aquatic organisms were found in the database and open access information resources consulted. No bibliographical records in relation to this subject were found. There is therefore no evidence for any adverse effects of hydrolysed proteins on aquatic ecosystems in general and on aquatic organisms in particular. *A priori*, hydrolysed proteins should be of low toxicological concern, since by definition they consist of the amino acid building blocks found in all living organisms and no risk to aquatic organisms is expected from their use as insect

attractant inplant protection products. Furthermore, it should be noted that the quantities of hydrolysed proteins (such as animal tissue, urea, collagen protein and beet molasses hydrolysates) that are used as spray bait to attract insects are very low in comparison to the amounts used in other applications (e.g in fertilizers).

No extra data are presenting only for a.s. urea below is the **ECHA endpoint summary** on Aquatic toxicity:

Urea is of very low acute toxicity to aquatic organisms.

CA 8.2.1 Acute toxicity to fish

No extra data are presenting only for urea below is the **ECHA endpoint summary**:

Toxicity to fish

The 48 hour LC50of urea in golden orfe is reported to be >10000 mg/l. This can also be considered as the NOEC. The results reported by the two laboratories were identical. The effects of urea on survival, food utilization and oxygen consumption of the fresh water fish Oreochromis mossambicus were studied. The percentage survival of O. mossambicus when exposed to different concentrations of urea at 24, 48, 72 and 96 h exposures was noted and it was found that 22000 and 38000 mg/L urea concentration were sublethal and lethal, respectively. The median lethal concentration, which killed 50% of the fish during 96 h exposure, was 28000 mg L−1. Rearing the fish in increasing sublethal concentrations of urea, it was found that the feeding rate decreased from 34.341 ± 7.067 mg g live fish−1 d−1(control) to 13.921 ± 2.315 mg g live fish−1 d−1at the highest concentration of urea (22,000 mg L−1). Growth rate was drastically reduced. The consumption of oxygen in O. mossambicus diminished from 0.962 ± 0.208 to 0.645 ± 0.118 mg g live fish−1 h−1 when reared in the highest sublethal concentration of urea. The 96 hour LC50of urea to B. barnawas > 9100 mg/l. The NOEL was 4961 ppm. The 96 hour acute LC50of urea to golden orfe fish is reported in a further study to be >6810 mg/l. No long-term toxicity data are available: a waiver is proposed for this endpoint. Urea is of inherently low toxicity to fish species: it is a normal product of protein catabolism and therefore fish have evolved effective excretion mechanisms. Additionally, exposure will be limited by the action of microorganisms and incorporation of urea into the nitrogen cycle.

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

CA 8.2.2.2 Fish full life cycle test

CA 8.2.2.3 Bioconcentration in fish

CA 8.2.3 Endocrine disrupting properties

CA 8.2.4 Acute toxicity to aquatic invertebrates

No extra data are presenting only for urea below is the **ECHA endpoint summary**:

Toxicity to aquatic invertebrates

The 24 hour EC50 for urea in Daphnia was reported to be >10000 mg/l; urea is not acutely toxic to daphnids. The 24 hour LC50 values for freshwater snail eggs, juveniles and adults were reported to be 14241 mg/l, 18255 mg/l and 22998 mg/l. Following 48 hours exposure, the LC50value for adults was calculated to be 13477 mg/l. In another study, the 24 hour LC50values for eggs, juvenile and adult snails were reported to be 13532 mg/l, 24504 mg/l and 26024 mg/l, respectively. Following 48 hours exposure, the LC50value for adults was calculated to be 21412 mg/l. It is concluded that, under normal laboratory conditions, urea displays low molluscicidal activity. The 4 hour LC50 in mosquito (Aedes aegypti) larvae is reported to be 60000 mg/l. No long-term toxicity data are available: a waiver is proposed on exposure grounds. Urea is of inherently low toxicity to species of aquatic invertebrates and exposure will be limited by the action of microorganisms and incorporation of urea into the nitrogen cycle

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

CA 8.2.5.4 Sediment dwelling organisms

CA 8.2.6 Effects on algal growth

No extra data are presenting only for urea below is the **ECHA endpoint summary**:

Toxicity to algae

The 192 hour toxicity threshold of blue-green algae urea was 47 mg/l. To some extent urea exhibits toxic action to Microcystis aeruginosa. The 7 day toxicity threshold of urea to Scenedesmus quadricauda was >10000 mg/l. The 72 hour toxicity threshold of Entosiphon sulcatum to urea was 29 mg/l, and the 16 hour toxicity threshold of urea to Pseudomonas putida was > 10000 mg/l.

CA 8.2.6.1 Effects on growth of green algae

CA 8.2.6.2 Effects on growth of an additional algal species

CA 8.2.7 Effects on aquatic macrophytes

CA 8.2.8 Further testing on aquatic organisms

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

CA 8.3.1.1.2 Acute contact toxicity

CA 8.3.1.2 Chronic toxicity to bees

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

CA 8.3.1.4 Sub-lethal effects

CA 8.3.2 Effects on non-target arthropods other than bees

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

CA 8.3.2.2 Effects on *Typhlodromus pyri*

CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna

CA 8.4.1 Earthworms – sub-lethal effects

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

CA 8.5 Effects on Nitrogen Transformation

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

CA 8.6.1 Summary of screening data

CA 8.6.2 Testing on non-target plants

CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

CA 8.8 Effects on Biological Methods for Sewage Treatment

CA 8.9 Monitoring Data

CA 8 ECOTOXICOLOGICAL STUDIES ON THE ACTIVE SUBSTANCE (SIC)

Introduction

The "Hydrolysed Protein" and its synonym "Protein Hydrolysate" is a multi meaning and not a single meaning term. It is a generic category term. One saying that the active substance is a "Protein Hydrolysate" he doesn't identify the substance but simply informs about the category of the substance.

Hydrolysed proteins have attractant properties, so they are indicated as raw materials for the elaboration of baits, and as a result, they increase the efficiency of the insecticide applied. The use of mass trappings helps to reduce plagues, as insects are retained inside the trap

The formulated product, whose components are the hydrolysed proteins is:
NUTREL containing "Hydrolysed Protein".

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.

CA 8.1 Effects on Birds and Other Terrestrial Vertebrates

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 Hydrolysed proteins 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 here below.

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

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 here below.

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)****

Exposure

Exposure of birds will be predominantly dietary, through the consumption of residues on food items. Direct exposure of birds to Hydrolysed proteins 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 Hydrolysed proteins 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 Hydrolysed proteins (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 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 following table.

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.

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 here below

Acute risk (TER_A) to birds 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 Hydrolysed proteins (NUTREL) at all proposed label rates in all crops.

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

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 Hydrolysed proteins (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 here below.

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 Hydrolysed proteins (NUTREL) presents no unacceptable long-term risk to birds when applied in all crops.

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 Hydrolysed proteins (NUTREL) in all crops.

CA 8.1.1 Effect on birds

CA 8.1.1.1 Acute oral toxicity to birds

CA 8.1.1.2 Short-term dietary toxicity to birds

CA 8.1.1.3 Sub-chronic and reproductive toxicity to birds

CA 8.1.2 Effects on terrestrial vertebrates other than birds

CA 8.1.2.1 Acute oral toxicity to mammals

CA 8.1.2.2 Long-term and reproductive toxicity to mammals

CA 8.1.3 Effects of active substance bioconcentration in prey of birds and mammals

CA 8.1.4 Effects on terrestrial vertebrate wildlife (birds, mammals, reptiles and amphibians)

CA 8.1.5 Endocrine disrupting properties

CA 8.2 Effects on Aquatic Organisms

Overview and summary

Effects on Aquatic Organisms

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

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 Hydrolysed proteins is not intended for direct application in water bodies.

Toxicity exposure ratios

The 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:

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

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

TER_A for Fish

In theory, the acute toxicity endpoint for Hydrolysed proteins 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.

Fish acute TER value (to 2 s.f.) 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	Hydrolysed proteins	N.A.	N.A.	N.A.	100

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

TER_{LT} for Fish

Hydrolysed proteins 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 Hydrolysed proteins poses low chronic risk to fish.

TER_A for Daphnia

In theory, the acute toxicity endpoint for Hydrolysed proteins 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.

Daphnia magna acute TER value for Hydrolysed proteins 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*	Hydrolysed proteins	N.A.	N.A.	N.A.	100

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

TER_{LT} for Daphnia

In theory, the long term Hydrolysed proteins 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 Hydrolysed proteins to all crops. The resulting TER_{LT} is presented below:

Long-term TER value (to 2 s.f.) for *D. magna* for Hydrolysed proteins

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 Hydrolysed proteins.

TER_A for Aquatic insect

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

TER_{LT} for Aquatic insect

In theory, the long term Hydrolysed proteins 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 Hydrolysed proteins to all crops. The resulting TER_{LT} is presented below:

Long-term TER value (to 2 s.f.) 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 Hydrolysed proteins.

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* indicated that Hydrolysed proteins poses low acute risk to aquatic invertebrates.

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* indicated that Hydrolysed proteins pose low long-term risk to aquatic invertebrates.

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* indicated that Hydrolysed proteins poses low acute risk to aquatic invertebrates. In addition, this data point is not relevant since Hydrolysed proteins L is not intended for use directly on surface waters.

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* indicated that Hydrolysed proteins poses low long-term risk to aquatic invertebrates. In addition, this data point is not relevant since Hydrolysed proteins L is not intended for use directly on surface waters.

TER_{LT} for Algae

In theory, the long-term risk to Algae from Hydrolysed proteins was assessed using the E_bC_{50} value for *Anabaena flos-aquae* 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.

Algae TER_{LT} value for Hydrolysed proteins

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 Hydrolysed proteins is above the Annex VI trigger value of 10, indicating that application of Hydrolysed proteins according to the proposed label uses poses low risk to algae.

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.

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.

CA 8.2.1 Acute toxicity to fish

CA 8.2.2 Long-term and chronic toxicity to fish

CA 8.2.2.1 Fish early life stage toxicity test

CA 8.2.2.2 Fish full life cycle test

CA 8.2.2.3 Bioconcentration in fish

CA 8.2.3 Endocrine disrupting properties

CA 8.2.4 Acute toxicity to aquatic invertebrates

CA 8.2.4.1 Acute toxicity to *Daphnia magna*

CA 8.2.4.2 Acute toxicity to an additional aquatic invertebrate species

CA 8.2.5 Long-term and chronic toxicity to aquatic invertebrates

CA 8.2.5.1 Reproductive and development toxicity to *Daphnia magna*

CA 8.2.5.2 Reproductive and development toxicity to an additional aquatic invertebrate species

CA 8.2.5.3 Development and emergence in *Chironomus riparius*

CA 8.2.5.4 Sediment dwelling organisms

CA 8.2.6 Effects on algal growth

CA 8.2.6.1 Effects on growth of green algae

CA 8.2.6.2 Effects on growth of an additional algal species

CA 8.2.7 Effects on aquatic macrophytes

CA 8.2.8 Further testing on aquatic organisms

CA 8.3 Effects on Arthropods

CA 8.3.1 Effects on bees

Summary and overview

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 Hydrolysed proteins 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 Hydrolysed proteins 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.

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 to bees of Hydrolysed proteins

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 Hydrolysed proteins. Overall the proposed use of Hydrolysed proteins is considered to pose a low risk to bees. No further data are required.

Hazard quotients for bees

No study were submitted.

In theory, the acute risk to honeybees from use of Hydrolysed proteins was assessed using the maximum single application rate and the lowest LD_{50} values (from the active substance) to calculate hazard quotients (ECPPO 2003)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 Hydrolysed proteins. A hazard quotient of less than 50 indicates a low risk to bees in the field.

Risk to bees from exposure to Hydrolysed proteins 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.

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 Hydrolysed proteins in all crops.

Oral exposure Q_{HO}

Reference is made to the above table

Contact exposure Q_{HC}

Reference is made to to the above table

Acute toxicity of the formulation to bees

Oral

No study were submitted.
The following bee acute and contact toxicity study performed on Hydrolysed proteins 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 10.4.2.1-1. 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.

Table 10.4.2.1-1: Bee mortality data for *Hydrolysed proteins*

Oral study (48 h)

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

Contact study (48 h)

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

Contact
Reference is made to point above.

Effects on bees of residues on crops

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

Cage tests

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

Field tests

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

Investigation into special effects

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

Tunnel tests

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

CA 8.3.1.1 Acute toxicity to bees

CA 8.3.1.1.1 Acute oral toxicity

CA 8.3.1.1.2 Acute contact toxicity

CA 8.3.1.2 Chronic toxicity to bees

CA 8.3.1.3 Effects on honeybee development and other honeybee life stages

CA 8.3.1.4 Sub-lethal effects

CA 8.3.2 Effects on non-target arthropods other than bees

Summary and overview

No study were submitted.

The following Arthropods Other Than Bees toxicity study performed on *Hydrolysed proteins* 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 *Hydrolysed proteins* 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 *Hydrolysed proteins* 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., 2000) and the EC Guidance Document on Terrestrial Ecotoxicology.

Risk assessment

The risk to non-target arthropods is assessed using the approach recommended in the ESCORT 2 guidance document (Candolfi et al., 2000) and the EC Guidance Document on Terrestrial Ecotoxicology.

In-field

In theory, the potential risk of *Hydrolysed proteins* 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 10.5-5.

Off-field

In theory, in order to assess the potential risk of *Hydrolysed proteins* 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:

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

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

Correction factor: ESCORT 2 (Candolfi et al., 2000) 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.

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.

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, *Hydrolysed proteins* poses low risk to in-field non-target arthropods following application according to the proposed use patterns.

CA 8.3.2.1 Effects on *Aphidius rhopalosiphi*

CA 8.3.2.2 Effects on *Typhlodromus pyri*

CA 8.4 Effects on Non-Target Soil Meso- and Macrofauna

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.

CA 8.4.1 Earthworms – sub-lethal effects

CA 8.4.2 Effects on non-target soil meso- and macrofauna (other than earthworms)

CA 8.4.2.1 Species level testing

CA 8.5 Effects on Nitrogen Transformation

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*

**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 nitrogen mineralizarion 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 *Hydrolysed proteins* because it is a soluble liquid formulation which mainly contains active ingredient and water.

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

Therefore, the application of *Hydrolysed proteins* poses low risk to micro-organisms.

CA 8.6 Effects on Terrestrial Non-Target Higher Plants

Effects on Non-Target Plants

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

Prelimary 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 *Hydrolysed proteins* for the vegetative vigour and the seedling emergence.

Other Non-Target Species (Flora and Fauna)

Tests on other non-target species are not required.

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

No data on other non-target species is required.

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

Use of *Hydrolysed proteins* 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.

CA 8.6.1 Summary of screening data

CA 8.6.2 Testing on non-target plants

CA 8.7 Effects on Other Terrestrial Organisms (Flora and Fauna)

Tests on other non-target species are not required.

CA 8.8 Effects on Biological Methods for Sewage Treatment

CA 8.9 Monitoring Data