SVMA14-004

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
21/02/2020	Additional data and information on aquatic organisms and harmful effects to non-target organisms in CP 10, CP 10.2 and CP 10.2.1 highlighted in yellow	SVMA14-004 document M-CP 10

[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 ECOTOXICOLOGICAL STUDIES ON PLANT PROTECTION PRODUCTS

SVMA14-004 is a representative formulation supporting the application for the renewal process of the active substance Hydrolysed Proteins in Europe. The critical use pattern is presented in the table below.

Table 10.1-1: Critical use pattern of the formulated product

Use No.	1	2
Crop	Citrus	Persimmon
Application rate (g as/ha)	450	450
Number of applications/minium interval	#	#
Crop growth stage (BBCH)	#	#
Application method	Foliar spray	Foliar spray

corresponds to the GAP of the insecticide used in mixture

In response to the RMS request, the applicant would like to highlight that Hydrolysed proteins are naturally occurring compounds whose degradation leads to simple metabolites called amino acids that are abundant organic molecules in living cells and used for the biosynthesis of new specific proteins and therefore essential for life.
Furthermore, in addition to the use as fertilisers as explained for environmental fate and behaviour section, hydrolysed proteins are also used as raw material for feed in aquaculture or animal feeding market.
On request, the applicant can provide several articles or examples of final products containing hydrolysed proteins in the market linked to the aquaculture and animal feeding uses, to reinforce this argumentation.
Moreover, the active substance hydrolysed proteins is not classified according to the ECHA.
The applicant is of the opinion that these information provide an argumentation strong enough to justify the exemption of further studies or risk assessments, considered as unnecessary from a scientific and rational point of view.

Moreover, for completeness purposes, the applicant provides a study performed with Hydrolysed proteins ([redacted], *"Bioefficacy of parentally-administered Norlan L 25 in piglets" (2004)* [redacted], and supplementary report) to prove that the administration of such compounds to non-target organisms has no harmful effects.
The conclusion of this study is that the use of Hydrolysed proteins resulted in significant increases in the concentration of monocytes and platelets and in mean platelet volume, but without having any significant effect on the growth and feed conversion index. Moreover, it was clearly highlighted that the increased concentration of monocytes observed after administration reveals an immune efficacy, inducing increased defences against various pathogenic microorganisms and therefore having non harful effects to non-target organisms.

CP 10.1 Effects on Birds and Other Terrestrial Vertebrates

CP 10.1.1 Effects on birds

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to birds and no testing toxicity data are required.

Risk assessment for birds

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to terrestrial vertebrates is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.1.1.1 Acute oral toxicity

No new data submitted, not required.

CP 10.1.1.2 Higher tier data on birds

No new data submitted, not required.

CP 10.1.2 Effects on terrestrial vertebrates other than birds

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.
No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to wild mammals and no testing toxicity data are required.

Risk assessment for other terrestrial vertebrates

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to terrestrial vertebrates is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.1.2.1 Acute oral toxicity to mammals

No new data submitted, not required.

CP 10.1.2.2 Higher tier data on mammals

No new data submitted, not required.

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

No new data submitted, not required.

CP 10.2 Effects on Aquatic Organisms

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to aquatic organisms and no testing toxicity data are required.

Risk assessment for aquatic organisms

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to aquatic organisms is expected following the use of SVMA14-004.

Nevertheless, the following assumption is presented instead. An unacceptable risk is identified when PEC/RAC > 1 and by taking into account the maximum PEC_{sx} of 23.550 µg/L calculated in Document M-CP 9, this would result in a RAC < 23.55 µg/L. This would happen if the corresponding acute LC_{50}/EC_{50} would be lower than 2.355 mg/L (with an AF of 100).
Such a low LC_{50}/EC_{50} is highly unexpected considering the nature of the active substance. In addition, it should be noted that according to the notifications provided to ECHA in REACH registrations and CLP notifications, no hazards have been classified for the active substance Hydrolysed proteins, referred as "*Protein hydrolyzates, animal*" by ECHA. Please refer to the summary from ECHA provided in Document M-CA 10.
Moreover, it should be noted that the review of the scientific literature within the last 10 years did not give any results indicating a hazardous effect or a potential risk for aquatic wildlife. Please refer to Document M-CA 9.

The evaluation of the risk for aquatic organisms was performed in accordance with the recommendations of the "Guidance document on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters in the context of Regulation (EC) No 1107/2009", as provided by the Commission Services (SANTE-2015-00080, 15 January 2015).
The relevant maximum instantaneous PEC_{SW} for risk assessments covering the proposed use patterns and the resulting PEC/RAC ratios are presented in the table below.

In the following table, the ratios between predicted environmental concentrations in surface water bodies (PEC_{SW}) and regulatory acceptable concentrations (RAC) for aquatic organisms are given for each intended use and each organism group.

Table 10.2-1: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for hydrolysed proteins for each organism group based on maximum instantaneous PEC_{SW} calculations for the use of SVMA14-004 in citrus early

Group		**Fish acute**	**Inverteb. acute**	**Algae**
Test species		***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***

Group		Fish acute	Inverteb. acute	Algae
Endpoint		EC_{50}	EC_{50}	EC_{50}
(µg/L)		> 100000	> 100000	313493
AF		100	100	100
RAC (µg/L)		> 1000	> 1000	3134.93
PEC_{SW} inst-max (µg/L)				
43.800		0.044	0.044	0.014

Table 10.2-2: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for hydrolysed proteins for each organism group based on maximum instantaneous PEC_{SW} calculations for the use of SVMA14-004 in persimmon early

Group		Fish acute	Inverteb. acute	Algae
Test species		***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint		EC_{50}	EC_{50}	EC_{50}
(µg/L)		> 100000	> 100000	313493
AF		100	100	100
RAC (µg/L)		> 1000	> 1000	3134.93
PEC_{SW} inst-max (µg/L)				
43.800		0.044	0.044	0.014

Table 10.2-3: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for hydrolysed proteins for each organism group based on maximum instantaneous PEC_{SW} calculations for the use of SVMA14-004 in citrus late

Group		Fish acute	Inverteb. acute	Algae
Test species		***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint		EC_{50}	EC_{50}	EC_{50}
(µg/L)		> 100000	> 100000	313493
AF		100	100	100
RAC (µg/L)		> 1000	> 1000	3134.93
PEC_{SW} inst-max (µg/L)				

Group		Fish acute	Inverteb. acute	Algae
23.550		0.024	0.024	0.008

Table 10.2-4: Aquatic organisms: acceptability of risk (PEC/RAC < 1) for hydrolysed proteins for each organism group based on maximum instantaneous PEC_{SW} calculations for the use of SVMA14-004 in persimmon late

Group		Fish acute	Inverteb. acute	Algae
Test species		***Danio rerio***	***Daphnia magna***	***Pseudokirchneriella subcapitata***
Endpoint (µg/L)		EC_{50} > 100000	EC_{50} > 100000	EC_{50} 313493
AF		100	100	100
RAC (µg/L)		> 1000	> 1000	3134.93
$PEC_{SW\ inst\text{-}max}$ (µg/L)				
23.550		0.024	0.024	0.008

Conclusion
For the intended uses on citrus (both early and late), calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive groups of aquatic organisms (risk for fish as characterised by an EC_{50} for *Danio rerio* of 100000.00 µg/L in connection with an assessment factor of 100 and for invertebrates as characterised by an EC_{50} for *Daphnia magna* of 100000.00 µg/L in connection with an assessment factor of 100) for all the instantaneous PECsw calculations. Therefore, no further assessment is necessary.

For the intended uses on persimmon (both early and late), calculated PEC/RAC ratios did indicate an acceptable risk for the most sensitive groups of aquatic organisms (risk for fish as characterised by an EC_{50} for *Danio rerio* of 100000.00 µg/L in connection with an assessment factor of 100 and for invertebrates as characterised by an EC_{50} for *Daphnia magna* of 100000.00 µg/L in connection with an assessment factor of 100) for all the instantaneous PECsw calculations. Therefore, no further assessment is necessary.

Therefore, this confirms that the use of Hydrolysed proteins as plant protection product is considered to pose a low risk to aquatic organisms.

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

According to the EFSA Conclusion (2012) a data gap was identified concerning toxicity studies for aquatic organisms: "*Studies on aquatic organisms that are necessary to fulfil the Annex II requirements directly related to classification and labelling*".
Therefore, and in agreement with the current data requirements related to Regulation (EU) 283/2013 the following toxicity studies have been submitted for aquatic organisms:

- Acute toxicity to fish (*Danio rerio*).
- Acute toxicity for aquatic invertebrates (*Daphnia magna*).
- Growth inhibition test for freshwater alga (*Pseudokirchneriella subcapitata*).

A summary of each new submitted study is available below.

Reference:	KCP 10.2.1-01
Report	ACUTE TOXICITY TEST ON AQUATIC ORGANISMS (Danio rerio) ON "NORLAN AMPL 201901001007", [redacted], 2020, report No. [redacted]
Guideline(s):	Yes, OECD 203
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	-

SVMA14-004 (called "NORLAN AMPL 201901001007" in the study) is a brown liquid formulation containing 300 g/L of animal proteins hydrolysate. The aim of the study is to determine the ecotoxicological effects of the test item on biotic systems: in particular aquatic organism such as Danio rerio fish in order to perform a test to define a limit not toxic threshold.
The toxicity of the test item, "NORLAN AMPL 201901001007", on the fish, Danio rerio, has been evaluated according to OECD guideline N. 203 concerning Acute toxicity test – LC_{50}.
In particular, the assay of acute toxicity with fish *Danio rerio* has been performed by means of a static assay in which the solution has not been changed for the whole test period. After a range finding test, a limit test has been performed using 14 fish, 7 of them treated with the test item at 100 mg/L and 7 fish used as control, kept in the same assay conditions without adding the test item.
Fish have been observed for a period of 96 hours and mortality and/or possible anomalous behaviours have been observed both in control and in treated.
Temperature, pH and percentage of saturation of oxygen in the water of each tank have been measured at the beginning of the treatment and with intervals of 24 hours.
Stability of the test item in the definitive test has been performed measuring organic carbon concentration at the beginning and at the end of the test in the tanks.

Material and methods

- **Assay conditions**

Water parameters at the beginning of the assay

To reduce the eventual presence of chlorine, the tap water used for the assay has been allowed to stabilize for 48 hours.
Tanks have been kept under control for temperature and oxygen for approximately 1 hour before introducing fish.
pH, temperature and oxygen have been measured at the beginning of the test in the water used for the experimentation.

Exposition

Animals have been added to the assay tanks with a biomass density lower than 0.8 g/L.
The treated group and the control group have been observed for 96 hours with a photoperiod of 12 hours of light and 12 hours of darkness.
Fish have been observed after 2 h ± 0.5h and 5 h ±1 h from the beginning of the test and twice per day until the end of the test.

- **Experimental design (Sample preparation)**

Range finding test

A solution at 1000 mg/L of the test item has been prepared in tap water. Then 5 different concentrations have been prepared with ten-fold dilutions from 100 mg/L to 0.01 mg/L.
The test has been performed on the diluted solutions.
N° of groups: 5 treated + 1 control
N° of animals : 3/tank

Definitive test (LC_{50} test)
After the range finding test a solution at 1000 mg/L has been prepared. Then the solution has been diluted to 100 mg/L in culture medium. The test has been performed on the diluted solution.
14 fish have been used for the definitive test subdivided as follows:

Group	Animals (N°)	Administered solution Nominal concentration	Observation times – Check point chemical-physical parameters (hours)
Control	7	Tap water	0-24-48-72-96
Treated	7	100 mg/L	0-24-48-72-96

N° of groups: 5 treated + 1 control
N° of animals : 7/tank

❖ **Determination of analytical concentration of total organic carbon (TOC)**
Determination of total organic carbon has been done using an automatic Shimadzu TOC analyser. It is a high sensitivity instrument based on the combustion catalytic oxidation method (680°C). The carbon dioxide generated by oxidation has been detected using an infrared gas analyser (NDIR). Samples, properly diluted with deionised water with low TOC contents, have been analysed by means of an integrated sampling system directly from vials containing 40 ml volume.
For each sample under test the corresponding control is analysed.

Results and discussion
Number of dead fish and symptomatology of fish in control and treated groups during the definitive test

Time / Group	2h	5h	1 day	1 day	2 day	2 day	3 day	3 day	4 day	4 day
Check N°	-	-	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7
Treated 100 mg/L	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7	0/0/7

x/x/x (number of death/number of abnormal changes or symptoms/number of fishes used)

❖ **Validity criteria**
The assay is considered valid if the following criteria are satisfied:
- 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% of the air saturation value throughout the test.
- Chemical conditions should be maintained during the test;

The validity criteria of the test are satisfied.
There are no mortality detected in control and treated animals.
There are no toxic symptoms detected in control and treated animals.
The temperature (24°C ± 1°C), % of oxygen saturation (above 60% both in control and in treated), pH (6.0-8.5) parameters as well of the stability of the test item (80-120%) are all satisfying the validity criteria.

Conclusion

The obtained results, in compliance with assay validity criteria, interpreted in accordance to OECD 203, showed that the *Danio rerio* LC_{50} of the test item "NORLAN AMPL 201901001007" after 96 hours is >100 mg/L.

Reference:	KCP 10.2.1-02
Report	ACUTE TOXICITY ON AQUATIC ORGANISMS (Daphnia sp.) ON "NORLAN AMPL 201901001007", C. Giarei, 2020, report No. STULV19AA4423-1 GLP
Guideline(s):	Yes, OECD 202
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	-

SVMA14-004 (called "NORLAN AMPL 201901001007" in the study) is a brown liquid formulation containing 300 g/L of animal proteins hydrolysate. The aim of the study is to determine the ecotoxicological effects of the test item "NORLAN AMPL 201901001007" on biotic systems: in particular on aquatic organism such as *Daphnia magna* in order to define a limit not toxic threshold according to the guideline OECD 202.
After a range finding test, the organisms have been exposed to the test item at a concentration of 100 mg/L for a total period of 48 hours.
Daphnie have been kept under observations for a period of 48 hours and the number of immobilised organisms and/or possible abnormal behaviours both in control and in treatment group vessel have been observed.
At the beginning and at the end of the test (48 hours) dissolved oxygen and pH have been measured in the highest concentration and in control group.
Temperature of the assay environment have been recorded at the beginning and at the end of the test.
Organic carbon concentration (TOC) has been measured at the beginning and at the end of the test in a pool derived from control and treated vessels.

Material and methods

- **Experimental design**

Medium preparation

As dilution water, reconstituted water has been used; it has been prepared dissolving the following salts in 1 litre of deionised water:

N. Solution	Reagents	Formula	Quantity (g/L)
A	Bihydrated calcium chloride	$CaCl_2\ 2H_2O$	11.76
B	Eptahydrated magnesium sulphate	$MgSO_4\ 7H_2O$	4.93
C	Acid sodium bicarbonate	$NaHCO_3$	2.59
D	Potassium chloride	KCl	0.23

25 mL of solution A, B, C and D have been taken to 1000 mL volume with deionised water.
Medium has been prepared according to the same proportion and reported on internal logbook.

Assay sample preparation

Range finding test

A solution at 1000 mg/L of the test item has been prepared in culture medium. Then 5 different concentrations have been prepared with ten-fold dilutions from 1000 mg/L to 0.10 mg/L. The test has been performed on the diluted solutions.
120 Daphnie have been used, 100 of them treated with the test item at different concentration and 20 used as control, kept in the same assay conditions without adding the test item.
At 24 e 48 hours immobilisation of the organisms has been evaluated.

Definitive test
After the range finding test, the test item has been prepared at 100 mg/L in culture medium. 40 Daphnie have been used, 20 of them treated with the test item at 100 mg/L and 20 used as control, kept in the same assay conditions without adding the test item, according to the design described in the following table.
At 24 e 48 hours immobilisation of the organisms has been evaluated.

Group	Replica N°	N° of individuals/replica	Administered Solution Nominal concentration	Observation times – Daphnia immobilization (hours)
Treated	4	5	100.00 mg/L	24-48
Control	4	5	Medium	24-48

Assay conditions
Lightning: None during the course of the test.
Feeding: None during the course of the test.
Temperature: 20°C ± 2°C
Water: Reconstituted dilution water
Dissolved oxygen: > 3 mg/L
Hardness: between 140 and 250 mg $CaCO_3$/L

- **Determination of analytical concentration of total organic carbon (TOC)**

Determination of total organic carbon has been done using an automatic SHIMADZU TOC analyser. It is a high sensitivity instrument based on the combustion catalytic oxidation method (680°C). The carbon dioxide generated by oxidation has been detected using an infrared gas analyser (NDIR). Samples, properly diluted with deionised water with low TOC contents, have been analysed by means of an integrated sampling system directly from vials containing 40 ml volume.
For each sample under test the corresponding blank has been analysed.

Results and discussion
Number of immobilised Daphnie at 24 and 48 hours in treated and control groups in the definitive control

Control	24 hours	48 hours
Control replication N.1	0/5	0/5
Control replication N.2	0/5	0/5
Control replication N.3	0/5	0/5
Control replication N.4	0/5	0/5
Concentration : 100.00 mg/L		
Treated -replication N.1	0/5	0/5
Treated -replication N.2	0/5	0/5
Treated -replication N.3	0/5	0/5
Treated -replication N.4	0/5	0/5

- **Validity criteria**

The assay is considered valid if the following criteria are satisfied:

- The immobilisation of control animals must not be higher than 10% at the end of the test.

- The concentration of dissolved oxygen in the vessels ought to be higher than 3 mg/L during the assay.
- However, in any case, the concentration of dissolved oxygen must not be lower than 2 mg/L.
- pH must not change more than 1.5 units.

The validity criteria of the test are satisfied.
There are no immobilisation detected in control and treated animals.
The temperature, oxygen concentration, pH parameters as well of the stability of the test item are all satisfying the validity criteria.

Conclusion
The obtained results, in compliance with assay validity criteria, showed that *Daphnia magna* EC_{50} after 48 hours of the nominal concentration of the test item "NORLAN AMPL 201901001007" is >100 mg/L.

Reference:	KCP 10.2.1-03
Report	FRESHWATER ALGA, GROWTH INHIBITION TEST ON "NORLAN AMPL 201901001007", C. Giarei, 2020, report No. STULV19AA4421-1 GLP
Guideline(s):	Yes, OECD 201
Deviations:	No
GLP:	Yes
Acceptability:	Yes/No/Supplementary
Duplication (if vertebrate study)	-

SVMA14-004 (called "NORLAN AMPL 201901001007" in the study) is a brown liquid formulation containing 300 g/L of animal proteins hydrolysate.
On the test item "NORLAN AMPL 201901001007" the growth inhibition test on a culture of algae Pseudokirchneriella subcapitata has been performed on a culture in exponential growth phase.
This approach, according to OECD 201, allowed to calculate the inhibition concentration for 50% of the organisms (IC_{50} or EC_{50}).
During the range finding test the algae have been exposed to 5 different dilutions of the test item for 72 hours. After the results of the range finding test an EC_{50} test has been performed. The algae have been exposed to 5 dilutions of the test item for 72 hours. Algae have been measured using a Burker chamber.
At the end of the test, microscopic observation was performed to verify a normal and healthy appearance of the inoculum culture and to observe any abnormal appearance of the algae.
At the beginning and at the end of the test (72 hours) pH has been measured in all the vessels.
Temperature of the ambient has been recorded every day.
Stability of the test item in the definitive test has been performed using TOC analyser. Organic carbon concentration has been measured at the beginning and at the end of the test in a pool derived from vessels at the higher, intermediate and lower tested concentration.

Materials and methods

❖ **Experimental design**

Culture medium
The culture medium has been prepared by mixing the 4 stock solutions in order to obtain final concentrations specified in the following table:

Solution N.	**Nutrient**	**Quantity dissolved in 500 ml of deionized water**

1	NH_4Cl	0,75 g
	$CaCl_2$ $2H_2O$	0,9 g
	$MgCl_2$ $6H_2O$	0,6 g
	$MgSO_4$ $7H_2O$	0,75 g
	KH_2PO_4	0,08 g
2	$FeCl_3$ $6H_2O$	0,032 g
	Na_2EDTA $2H_2O$	0,05 g
3	H_3BO_3	0,0925 g
	$MnCl_2$ $4H_2O$	0,207 g
	$ZnCl_2$	1 mL of the following solution: 150 mg in 100 mL of deionized water
	$CoCl_2$ $6H_2O$	1 mL of the following solution: 75 mg in 100 mL of deionized water
	$CuCl_2$ $2H_2O$	1 mL of the following solution: 25 mg in 500 mL of deionized water, then diluting 1 mL of it in 10 mL
	Na_2MoO_4 $2H_2O$	1 mL of the following solution: 35 mg in 10 mL of deionized water
4	$NaHCO_3$	25 g

Solutions 1, 2, 3 have been sterilised by autoclaving (120°C, 15 min). Solution 4 has been sterilised via membrane filtration (0.22 mm). 10 mL of solution 1 and 1 mL of solutions 2, 3, 4 have been made up to 1000 mL with deionised water.
For higher volumes the medium has been prepared according to the same proportion.

❖ **Sample preparation**

Range finding test

A solution at 1000 mg/L of the test item has been prepared in culture medium. Then 5 different concentrations have been prepared, with ten-fold dilutions from 1000 mg/L to 0.1 mg/L. The test has been performed on the diluted solutions.

Definitive test

After the range finding test a solution at 2000 mg/L of the test item has been prepared in culture medium. The test has been performed on the diluted solutions. (see Table below). The total volume of the culture medium and the concentration of the algae inoculum added were the same in all vessels.

Group	**Number**	**Cellular density (cells/ml)**	**Administered solution Nominal concentration**	**Cell observation times (hours)**
Treated 1	3	10^4	1700.00 mg/L	0-24-48-72
Treated 2	3	10^4	531.25 mg/L	0-24-48-72
Treated 3	3	10^4	166.02 mg/L	0-24-48-72
Treated 4	3	10^4	51.88 mg/L	0-24-48-72
Treated 5	3	10^4	16.21 mg/L	0-24-48-72
Control	6	10^4	Culture Medium	0-24-48-72

❖ **Determination of organic carbon for analytical verification**

Determination of Total Organic Carbon (TOC) has been done using an automatic SHIMADZU TOC analyser. It is a high sensitivity instrument based on the combustion catalytic oxidation method (680°C). The carbon dioxide generated by oxidation has been detected using an infrared gas analyser (NDIR). Samples, properly diluted with deionised water with low TOC contents, have been analysed by means of an integrated sampling system directly from vials containing 40 mL volume. For each sample, the corresponding control has been analysed.

Results and discussion

% inhibition of growth rate

Parameter	Treated sample				
	1700.00 mg/L	531.25 mg/L	166.02 mg/L	51.88 mg/L	16.21 mg/L
%I_r	68.80	47.65	45.99	39.83	19.14

❖ **Validity criteria**

The assay is considered valid if the following criteria are satisfied:

- the cell concentration in the control cultures should have increased by a factor of at least 16 within three days corresponds to a specific growth rate of 0.92 day -1.
- the mean coefficient of variation for section by section (CVss) specific growth rates in the control cultures must not exceed 35%.
- the coefficient of variation of average specific growth rates during the whole period (CVwp) in replicate control cultures must not exceed 7%.

The validity criteria of the test are satisfied.
A normal and healthy appearance of the algae at the end of the test was observed at microscopic for control and treated group.
The temperature and pH parameters as well of the stability of the test item are all satisfying the validity criteria.

Conclusion

The obtained results, in compliance with assay validity criteria, showed that Algae EC_{50} of the concentration of the test item "NORLAN AMPL 201901001007" after 72 hours is 313.493 mg/L.

The endpoints drawn from these studies are summarized in the table below and will be used for risk assessment.

Table 10.2-5: Endpoints and effect values relevant for the risk assessment for aquatic organisms – Hydrolysed proteins

Species	Substance	Exposure System	Results	Reference
Danio rerio	SVMA14-004	96h, s	EC_{50} > 100 mg/L	[redacted], 2019, Report No [redacted]
Daphnia magna	SVMA14-004	48h	EC_{50} > 100 mg/L_{nom}	C. Giarei, 2019, Report No STULV19AA4423-1 GLP
Pseudokirchneriella subcapitata	SVMA14-004	72h	EC_{50} = 313.493 mg/L	C. Giarei, 2019, Report No STULV19AA4421-1 GLP
Higher-tier studies (micro- or mesocosm studies)				
No data				

s: static; ss: semi-static; f: flow-through; nom: based on nominal concentrations; mm: based on mean measured concentration
Please note that endpoints for the formulation SVMA14-004 are the same than those for the active substance Hydrolysed proteins due to the composition of the formulation SVMA14-004 (please refer to Part C).

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

No data submitted, not required. Please refer to point 10.2.

CP 10.2.3 Further testing on aquatic organisms

No data submitted, not required. Please refer to point 10.2.

CP 10.3 Effects on Arthropods

CP 10.3.1 Effects on bees

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to bees and no testing toxicity data are required.

Risk assessment for bees

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to bees is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.3.1.1 Acute toxicity to bees

CP 10.3.1.1.1 Acute oral toxicity to bees

No new data submitted, not required.

CP 10.3.1.1.2 Acute contact toxicity to bees

No new data submitted, not required.

CP 10.3.1.2 Effects on honey bee development and other honey bee life stages

No new data submitted, not required.

CP 10.3.1.3 Sub-lethal effects

No new data submitted, not required.

CP 10.3.1.4 Cage and tunnel tests

No new data submitted, not required.

CP 10.3.1.5 Field tests with honeybees

No new data submitted, not required.

CP 10.3.2 Effects on non-target arthropods other than bees

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to non-target arthropods and no testing toxicity data are required.

Risk assessment for other non-target arthropods

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to non-target arthropods is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.3.2.1 Standard laboratory testing for non-target arthropods

No new data submitted, not required.

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

No new data submitted, not required.

CP 10.3.2.3 Semi-field studies with non-target arthropods

No new data submitted, not required.

CP 10.3.2.4 Field studies with non-target arthropods

No new data submitted, not required.

CP 10.3.3 Other routes of exposure for non-target arthropods

No new data submitted, not required.

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

CP 10.4.1 Earthworms

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to earthworms and no testing toxicity data are required.

Risk assessment for earthworms

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to earthworms is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.4.1.1 Earthworms – sub-lethal effects

No new data submitted, not required.

CP 10.4.1.2 Earthworms – field studies

No new data submitted, not required.

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

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to other soil-macroorganisms and no testing toxicity data are required.

Risk assessment for other non-target soil meso- and macrofauna (other than earthworms)

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to other soil-macroorganisms is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.4.2.1 Species level testing

No new data submitted, not required.

CP 10.4.2.2 Higher tier testing

No new data submitted, not required.

CP 10.5 Effects on Soil Nitrogen Transformation

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to soil microorganisms and no testing toxicity data are required.

Risk assessment for Soil Nitrogen Transformation

No risk assessment performed, not required. No adverse effect of Hydrolysed proteins to soil microorganisms is expected following the use of SVMA14-004.

Please refer to the argumentation stated in point B10.

CP 10.6 Effects on Terrestrial Non-Target Higher Plants

No new data submitted, not required.
SVMA14-004 is not intended to be used as an herbicide or a plant growth regulator, and is not known to have any herbicidal activities.

No EU endpoints available for the active substance.

Due to the nature of the Hydrolysed proteins and their characteristics, their use as plant protection product is considered to pose a low risk to non-target plants and no testing toxicity data are required.

Please refer to the argumentation stated in point B10.

CP 10.6.1 Summary of screening data

No new data submitted, not required.

CP 10.6.2 Testing on non-target plants

No new data submitted, not required.

CP 10.6.3 Extended laboratory studies on non-target plants

No new data submitted, not required.

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

No new data submitted, not required.

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

No new data/study with the formulation SVMA14-004 was performed, since according to the composition of the product (please refer to Document J) it is possible to extrapolate from data obtained with the active substance.

CP 10.8 Monitoring Data

No data submitted, not required.
It should be noted that since proteins are naturally occurring in the environment, it would not be possible to distinguish the naturally compounds from those resulting from the use of plant protection products. Thus, the concept of environmental monitoring is not applicable to Hydrolysed proteins.