Hydrolysed Proteins

DOCUMENT M-CA, Section 7

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Version history[1]

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21/02/2020	Additional information on aerobic biodegradability and vapour pressure/DT50 in air in CA 7.2.2.1 and CA 7.3.2 highlighted in yellow	Hydrolysed Proteins document M-CA 7

[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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CA 7 FATE AND BEHAVIOUR IN THE ENVIRONMENT

Hydrolysed proteins are naturally occurring compounds of degradation from the hydrolysis of living organisms' tissues that can have vegetable or animal origin. The degradation of the hydrolysed proteins results in more simple metabolites called amino acids. Proteins and amino acids are abundant organic molecules in living cells. They can be found in every single cell, since they are fundamental in all aspects of the cell structure and function, and intervene in the most essential biochemical processes.
The proteins are one of the three basic principal nourishment of living beings. The proteins that are found in food and eaten by human beings and mammals are normally degraded metabolically by means of enzymatic processes and results in amino acids, that are then used by the living cells for the biosynthesis of new specific proteins.
Thus, the hydrolysed proteins are biodegradable, so their persistence in the environment is very short without any tendency for bioaccumulation.
Due to the nature of the hydrolysed proteins and their characteristics regarding the fate and behaviour in the environment, it is deemed very unlikely the existence of relevant residues resulting from applications as plant protection product in the soil, surface water or sediment and even more unlikely the existence of relevant residues reaching the groundwater.

For all these reasons, it is deemed not necessary to conduct any studies with Hydrolysed proteins about the fate and behaviour in the environment.

In this context, Hydrolysed Proteins meet the criteria for the approval of low-risk active substance because not considered to be persistent nor to have potential for bioaccumulation.

CA 7.1 Fate and Behaviour in Soil

CA 7.1.1 Route of degradation in soil

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.1.1 Aerobic degradation

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.1.2 Anaerobic degradation

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.1.3 Soil photolysis

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2 Rate of Degradation in Soil

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2.1 Laboratory studies

CA 7.1.2.1.1 Aerobic degradation of the active substance

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2.1.2 Aerobic degradation of metabolites, breakdown and reaction products

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2.1.3 Anaerobic degradation of the active substance

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2.1.4 Anaerobic degradation of metabolites, breakdown and reaction products

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2.2 Field Studies

CA 7.1.2.2.1 Soil dissipation studies

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.2.2.2 Soil accumulation studies

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.3 Absorption and desorption in soil

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.3.1 Adsorption and desorption

CA 7.1.3.1.1 Adsorption and desorption of the active substance

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.3.1.2 Adsorption and desorption of metabolites, breakdown and reaction products

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.3.2 Aged sorption

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.4 Mobility in soil

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.4.1 Column leaching studies

CA 7.1.4.1.1 Column leaching of the active substance

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.4.1.2 Column leaching of metabolites, breakdown and reaction products

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.4.2 Lysimeter studies

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.1.4.3 Field leaching studies

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2 Fate and Behaviour in Water and Sediment

CA 7.2.1 Route and rate of degradation in aquatic systems (chemical and photochemical degradation)

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.1.1 Hydrolytic degradation

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.1.2 Direct photochemical degradation

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.1.3 Indirect photochemical degradation

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.2 Route and rate of biological degradation in aquatic systems

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.2.1 "Ready biodegradability"

In response to the RMS request regarding "Ready biodegradability" of Hydrolysed proteins, the applicant PROALAN S.A. has performed a study to assess the aerobic biodegradability of the product SVMA14-004. Furthermore, it is important to note that due to the composition of the product SVMA14-004, the results of this study can be extrapolated to the active substance Hydrolysed proteins.

A summary of this study is provided below.

Reference:	KCA 7.2.1
Report	C. Giarei (2019) : EVALUATION OF AEROBIC BIODEGRADABILITY ON "NORLAN AMPL 201901001007", Report No. STULV19AA4428-1 GLP
Guideline(s):	OECD 310:2014. Ready Biodegradability - CO_2 in sealed vessels (Headspace Test)
Deviations:	No
GLP:	Yes
Acceptability:	Yes
Duplication (if vertebrate study)	Not relevant

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 analysis of inorganic carbon for the evaluation in an aqueous medium of the aerobic biodegradability has been performed following screening method described in OECD 310: 2014.
For this purpose the amount of developed inorganic carbon has been measured and reported in comparison to the blank. The test has been done using the test item with a fixed concentration of organic carbon. Samples have been kept at the temperature of 20 ± 1 °C for the whole period of the test (28 days).

Material and method

- **Mud sampling and preparation**

Biodegradability of a substance is based on different parameters and, among them, composition and concentration of bacterial biomass play an important role.
For the preparation of the inoculum a sample of aerobic sludge has been selected by the mixed treatment plant of urban (about 66%) and industrial (about 34%) liquid sewage situated at San Rocco - Monza (MB), Italia. The plant of treatment is managed by "BRIANZACQUE SRL (Monza)".

In the laboratory, the sampled muds have been mixed and let settle, keeping them in aerobic conditions for 4 days.
The mud samples, before their use, have been analysed to check its ability to form colony units.
Then it has been centrifuged, washed and analyzed to quantify the suspended solids concentration for the inoculum preparation. The inoculum was prepared in order to have a concentration of suspended solid of about 4 mg/L.

- **Medium preparation**

Solution A

	Formula	Concentration (g/L)
Anhydrous potassium dihydrogenphosphate	KH_2PO_4	8.50
Anhydrous dipotassium hydrogenphosphate	K_2HPO_4	21.75
Disodium hydrogenphosphate dihydrate	$Na_2HPO_4.2H_2O$	33.40
Ammonium chloride	NH_4 CI	0.50

Solution B

	Formula	Concentration (g/L)
Calcium chloride dihydrate	$CaCl_2$ $2H_2O$	36.40

Solution C

	Formula	Concentration (g/L)
Magnesium sulfate heptahydrate	$MgSO_4$ $7H_2O$	22.50

Solution D

	Formula	Concentration (g/L)
Iron chloride (III) hexahydrate	$FeCl_3$ $6H_2O$	0.25

All solutions have been made with deionized water (MilliQ Millipore).
Then for each litre of medium 10 mL of Solution A have been transferred into a 1000 mL volumetric flask and diluted with 800 mL of deionised water; 1 mL of Solution B, 1 mL of Solution C and 1 mL of Solution D have been added and the solution has been diluted to volume with deionised water. For higher volumes the medium has been prepared according to the same proportion. The preparation of the solutions has been reported in an internal logbook.

- **Bottle preparation**

Bottles have been used as follows:

Bottles type	N. of total Replica prepared	N. of total Replica used	N. Replica/ Check points (days)
Blank	23	17	3 replica / once a week 5 replica at 28th day
Test sample	23	17	3 replica / once a week 5 replica at 28th day
Reference substance	23	17	3 replica / once a week 5 replica at 28th day
Test sample + Reference substance	8	8	3 replica /t=1st day 5 replica at 28th day

Abiotic check	8	8	3 replica /t=1st day 5 replica at 28th day

to which the inoculum of the muds has been added. All dilutions have been done using deionised water.

For assay execution the following preparations have been done using deionised water.
Bottles not used have been eliminated at the end of the test.

Test sample
The elemental analysis of the test item has been determined by REDOX SnC using CHN analyser. The sample has been used with the starting nominal concentration of 20.05 mg/L of organic carbon (TOC).
Reference substance
Considering Sodium benzoate as reference substance using its molecular formula, a starting nominal concentration of 20.02 mg/L of organic carbon (TOC) has been used.
Blank
Blank has been used which contained water, culture medium and the inoculum of the mud.
Test sample + Reference substance
Test sample and reference substance have been put together in order to have a final concentration given by the sum of organic carbon (TOC) of the 2 substances.
Abiotic check
Test item has been put together with 50 mg/L $HgCl_2$ in order to check a possible abiotic degradation.

❖ **Conditions of assay**

The inoculum has been added in all bottles, closed and incubation continued under stirring in darkness at 20 ± 1 °C for 28 days. Data recording is performed continuously by a validated informatic monitoring system (Labguard Evisense).
At least 1 hour before every determination of inorganic carbon (TIC) the reaction in one bottle of blank, one of reference substance and one of the test item has been stopped with the addition of 6 mL of sodium hydroxide (1 M).
Triplicate bottles have been analyzed to detect inorganic carbon (IC) at each check point, weekly, except at the end of the test where five bottles have been analyzed.

❖ **Determination of total organic carbon (TOC) and total inorganic carbon (TIC)**

Determination of total inorganic carbon has been done using an automatic 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 analyzer (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.

Results and discussion

TIC T=1 day (ppm)	TIC T=28 day (ppm)	% INCREASE (<10%)	CONFORMITY
3,400	3,569	4,960	CONFORM

❖ **Validity criteria and interpretation of results**

The test is considered valid when:

- the percentage of average degradation of the reference substance is more than 60% after 14 days of incubation.

- the mean amount of TIC present in the blank at the end of the test is < 3 mg C/L

If, at day 28, the biodegradation in the bottle contained both the test sample and the reference substance referred to biodegradation in the bottle with reference substance result < 25%, it may be assumed that the test substance doesn't inhibit the activity of the inoculum. A substance is considered readily biodegradable when its level of biodegradability is at least 60%, within a 10d window during the test.

Validity criteria of the test are satisfied.
The mean amount of TIC present in the blank controls at the end of the test is < 3 mg C/L.
Abiotic degradation has not occurred because amount of TIC during the test in the abiotic bottles has been lower than 10%.
The trend of the inorganic carbon and related biodegradation percentages (see table below - column % of biodegradation Reference substance + test item) in the bottle contained both the test sample and the reference substance confirms the absence of an inhibitory effect of the test sample on inoculum at the concentration at which it was applied in the test (8%).

CHECK POINT (Days)	% OF BIODEGRADATION Reference substance	% OF BIODEGRADATION Test item	% OF BIODEGRADATION Reference substance+ Test item
0	0	0	0
1	34	23	45
7	78	73	-
14	77	75	-
21	77	70	-
28	75	69	69

❖ **Conclusion**

On the basis of results obtained, interpreted in accordance to OECD 310:2014, the test item "NORLAN AMPL 201901001007" is considered readily biodegradable in aerobic conditions.

CA 7.2.2.2 Aerobic mineralisation in surface water

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.2.3 Water/sediment studies

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.2.4 Irradiated water/sediment study

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.2.3 Degradation in the Saturated Zone

No data submitted, not required. Please refer to the argumentation presented under Point B 7.

CA 7.3 Fate and Behaviour in Air

The active substance Hydrolysed Proteins is a natural substance biodegradable. Some of the metabolites resulting from the decomposition process are volatiles substances, such as ammonia, carbon dioxide or putrescine.

CA 7.3.1 Route and rate of degradation in air

No data submitted, not required. The volatile compounds from Hydrolysed proteins are naturally occurring compounds of no concern.

CA 7.3.2 Transport via air

In response to the RMS request, the applicant wants 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.
Furthermore, amino acids from hydrolysis of animal proteins are used as fertilisers in Spain according to the Real Decreto 506/2013 (please refer to p 35, group 4.1 called “Aminoácidos”). In addition, hydrolysed proteins of category 3 materials is part of the proposal of the future new EU regulation for fertilisers products (please refer to the proposal of December, the 4th, page 83)
Therefore, these compounds are considered to be of low risk for soil and water compartments and considering that they are widely used as fertilisers in Europe, the amount of hydrolysed proteins added to the environmental compartments linked to the application as plant protection product is considered to be negligible in comparison to the amounts derived from the use as fertilisers.

The applicant is of the opinion that these information provide an argumentation strong enough to justify the exemption of further studies, considered as unnecessary from a scientific and rational point of view.

However, for completeness purposes, the applicant provides new data for vapour pressure and half-life in air for amino acids.
The new data are drawn from open literature (*Clyde, Dale Dean and Svec, Harry, "Vapor pressures of some amino acids" (1963). Ames Laboratory Technical Reports. 51.*) and from the information available on PubChem, ChemIDplus, ChemicalBook, and on the basis of AOPWin estimations.

These new data on vapor pressure found in open literature are presented below:

Amino acids	Temperature (°k)	Pressure (mm Hg)
Glycine	453	0.0587
	457	0.0859
	466	0.159
	471	0.243
l-alanine	453	0.0759
	460	0.122

	465	0.203
	469	0.258
1-α-amino-n-butyric acid	449	0.0972
	452	0.1290
	455	0.163
	462	0.360
dl-norvaline	439	0.0404
	446	0.0664
	452	0.1010
	461	0.1930
1-valine	438	0.0395
	444	0.0682
	448	0.103
	452	0.150
	456	0.233
1-leucine	464	0.216
	454	0.0936
	452	0.0844
	446	0.0440
1-methionine	463	0.0384
	472	0.0622
	478	0.105
	485	0.163
1-phenylalanine	451	0.0252
	457	0.0463
	463	0.0758
	469	0.119
1-proline	442	0.0675
	448	0.107
	451	0.171
	457	0.210
	465	0.307
	467	0.299
dl-nor1eucine	435	0.0190
	449	0.0576
	461	0.129
	469	0.184
isoleucine	442	0.0763
	448	0.106
	453	0.172
	456	0.209
	461	0.262
cycloleucine	443	0.0666
	450	0.112
	456	0.166
	462	0.269

	468	0.351
α-amino isobutyric acid	462	0.451
	452	0.218
	441	0.108
	439	0.078

These new data on vapour pressure found on PubChem, ChemIDplus, and ChemicalBook are presented below:

Amino acids	**Vapour pressure (mm Hg at 25°C)**	**Source**	**Original source**
Aspartic acid	2.60 x 10^{-7}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Oct 26, 2006*)
Glutamic acid	1.70 x 10^{-8}	PubChem	EPA DSSTox
Serine	4.02 x 10^{-8}	ChemIDplus	SRC
Histidine	5.99 x 10^{-9}	ChemIDplus	SRC
Glycine	1.28 x 10^{-7}	ChemicalBook	-
Threonine	1.32 x 10^{-8}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from, as of Feb 22, 2010*)
Alanine	1.05 x 10^{-7}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver.3.12. Nov 30, 2004. Available from, as of Sept 5, 2006*)
Arginine	2.10 x 10^{-6}	ChemicalBook	-
Cysteine	6.73 x 10^{-7}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.1. Nov, 2012. Available from, as of July 12, 2016*)
Valine	5.55 x 10^{-9}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from, as of Feb 22, 2010*)
Methionine	8.14 x 10^{-8}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from, as of Feb 19, 2010*)
Phenylalanine	1.76 x 10^{-8}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from, as of Feb 16, 2010*)
Isoleucine	6.85 x 10^{-9}	PubChem	EPA (*US EPA; Estimation Program Interface (EPI) Suite. Ver. 4.0. Jan, 2009. Available from, as of Feb 22, 2010*)
Leucine	1.34 x 10^{-8}	ChemIDplus	SRC
Lysine	5.28 x 10^{-9}	PubChem	HSDB (*Daubert, T.E., R.P. Danner. Physical and Thermodynamic Properties of Pure Chemicals Data Compilation. Washington, D.C.: Taylor and Francis, 1989*)
Proline	3.77 x 10^{-9}	ChemIDplus	SRC

New data on half-life in air found on PubChem and based on AOPWin estimations are presented below:

Amino acids	DT_{50} (Half-life in hours)	Source	Original source
Aspartic acid	10	PubChem	SRC
Glutamic acid	9.4	PubChem	SRC
Glycine	4.578	-	AOPWin v. 1.92a (Sept 2010) estimation (*EPISuite 4.1; US EPA*)
Alanine	11	PubChem	SRC
Arginine	0.946	-	AOPWin v. 1.92a (Sept 2010) estimation (*EPISuite 4.1; US EPA*)
Valine	1.6	PubChem	SRC
Phenylalanine	7.5	PubChem	SRC
Isoleucine	8.7	PubChem	SRC
Lysine	3.034	-	AOPWin v. 1.92a (Sept 2010) estimation (*EPISuite 4.1; US EPA*)

CA 7.3.3 Local and global effects

According to its composition, the active substance Hydrolyse Proteins contains a significant content of nitrogen, and therefore the eutrophication potential must be assessed.
Please refer to the calculations performed in Section MCP-9, point 9.2.5.

CA 7.4 Definition of the Residue

CA 7.4.1 Definition of the residue for risk assessment

Soil: Animal tissue hydrolysate
Groundwater: Animal tissue hydrolysate
Surface water/sediment: Animal tissue hydrolysate
Air: Animal tissue hydrolysate

CA 7.4.2 Definition of the residue for monitoring

No residue definition for monitoring, not required. Please refer to the argumentation presented under Point B 7.
Furthermore, it should be noted that since proteins are naturally occurring in the environment, it would not possible to distinguish the naturally compounds from those resulting from the use of plant protection products.

CA 7.5 Monitoring Data

No data submitted, not required.

It should be noted that since proteins are naturally occurring in the environment, it would not 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 for Hydrolysed proteins.