BIOCEBO/BIO

DOCUMENT M-CP, Section 9

FATE AND BEHAVIOUR IN THE ENVIRONMENT

Version history[1]

Date	Data points containing amendments or additions and brief description	Document identifier and version number
November 2019	CP 9.1.3 and CP 9.2: new PECs, PECsw and PECgw calculations are provided.	M-CP Section 9 - BIOCEBO version 2

[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 9 FATE AND BEHAVIOUR IN THE ENVIRONMENT

According to the document Draft Assessment Report (DAR), hydrolysed proteins are natural compounds of degradation from the hydrolysis of living organisms 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.

The hydrolysed proteins are biodegradable, so their persistence in the environment is very short, without existing any tendency to bioaccumulation.

Due to the nature of the active substance (hydrolysed proteins), the type of use of BIOCEBO and the characteristics of the active substance regarding its fate and behaviour in the Environment, it could be considered very unlikely the existence of relevant residues of the active substance in the soil derived from the application of BIOCEBO. In addition, it is unlikely that leaching of the active substance can occur or that residues can reach groundwater under the proposed conditions of use. For this reason, it is not necessary to carry out the evaluation of the fate and behaviour in the environment of BIOCEBO.

Predicted environmental concentrations (PEC values) in soil, surface water, sediment, groundwater and air are null, provided that BIOCEBO is used according to GAP. The Reference Member State evaluation in the Hydrolysed Proteins DAR considers that a satisfactory justification was provided for not determining Hydrolysed Proteins residues in soil, water, air, body fluids and tissues. No data gap was reported on this aspect in the Assessment Report.

The justifications are based on results obtained for the active substance (as) contained in the formulation. Full details of all proposed uses pattern that will be assessed are summarised in following table.

Critical use pattern of AL (Other liquids to be applied undiluted)

Use	Application rate (g as/ha)	Application method	Number of applications	Minimum application interval (days)	Application timing
Attractant	450 g as/ha	Patch spray	1.50 L of BIOCEBO per 100L of water.	Not applicable	Fruit developement, (BBCH80-BBCH90)

The impact of formulants is limited to short-term effects such as stabilising the odour emission of the attractant, to make it evident to target organisms. Therefore, for the purposes of this risk assessment it is assumed that formulants do not influence the fate and behaviour of the active substance in the environment and are not considered further.

CP 9.1 Fate and Behaviour in Soil

CP 9.1.1 Rate of degradation in soil

A GLP ready biodegradability study (Modified Sturm Test) according to the OECD 301 B Guideline (CO_2 evolution test) has been performed by Noack Laboratorien. Based on personal communication with the study director, it was confirmed that the test item is 'readily biodegradable' according to the criteria specified in the OECD guideline. At the time of writing of this update, the final report of the OECD 301 B study was not yet available (foreseen March 2020). However, in the PEC calculations presented below, ready biodegradability was already assumed.

CP 9.1.1.1 Laboratory studies

Not applicable.

CP 9.1.1.2 Field studies

The field dissipation rates of Hydrolysed Proteins were not evaluated during the Annex I inclusion. ~~For the product, if applied under GAP, there would be not contact between the product and the soil.~~ No additional studies have been performed.

Conclusion/endpoint: no significant risk could be expected from the correct application of the product BIOCEBO.

CP 9.1.1.2.1 Soil dissipation studies

Not available.

CP 9.1.1.2.2 Soil accumulation studies

It will be impossible to distinguish between the product residues and the decomposition residues of any other living organism. The same degradation products could be expected.

CP 9.1.2 Mobility in soil

The mobility of Hydrolysed Proteins was not evaluated during the Annex I inclusion. For the product, if applied under GAP, there would not be contact between the product and the soil. No additional studies have been performed.

Conclusion/endpoint: no significant risk could be expected from the product BIOCEBO.

CP 9.1.2.1 Laboratory studies

Not applicable.

CP 9.1.2.2 Lysimeter studies

Not applicable.

CP 9.1.2.3 Field leaching studies

Not applicable.

CP 9.1.3 Estimation of concentrations in soil

Predicted environmental concentrations in soil (PECs)

The PECs is calculated for the active substance 'hydrolysed proteins' for the critical use in fruit trees and olives (lowest crop interception value, cf. Table 9.1.3-1).

Table 9.1.3-1: Identification of critical use for PEC_{soil} calculations (in bold)

Crop	Dose rate (g hydrolysed protein/ha)	Crop interception (%)	Dose reaching soil (g hydrolysed protein/ha)
Fruit trees, olive	3 x 450	65	3 x 157.5
Citrus	3 x 450	80	3 x 90

CP 9.1.3.1 Active substance(s) and relevant metabolite(s)

The PECs calculations for hydrolysed protein are performed according to FOCUS guidelines. Tables 9.1.3-2 and 9.1.3-3 show the input parameters that were used in the calculation.

Table 9.1.3-2: Input parameters related to application for PEC_{soil} calculations

Crop	Fruit trees, citrus, olive
Application rate (g as/ha)	hydrolysed protein: 450
Number of applications/interval	Max. 3 applications with a min. interval of 7 days
Crop interception (%)	Interception values according to Appendix C of the EFSA Guidance to obtain $DegT_{50}$ values (2014): Apple: 65% (BBCH≥71) Citrus: 80% (all BBCH stages) → worst-case interception of 65% is used
Depth of soil layer (cm)	5

Table 9.1.3-3: Input parameter for the active substance for PEC_{soil} calculations

Compound	DT50 (days)	Value in accordance to EU endpoint y/n/ Reference
Hydrolysed protein	30	Preliminary results of the biodegradability test (OECD 301/310) showed that the active substance is readily biodegradable. Default DT_{50} value for readily biodegradable substances with a solid-water partition coefficient in soil (Kpsoil) <100 l/kg, in accordance with ECHA's Guidance on information requirements and Chemical Safety Assessment, Chapter R.16: Environmental exposure assessment (Table R.16-12 for soil). Note: Kpsoil can be calculated as Foc_{soil} x Koc, with Foc_{soil}=0.02 (default value) and Koc=10 (default value; worst case value when considering KOWWIN v. 1.68 (EPI Suite) QSAR predictions of octanol-water partition coefficients for amino acids and peptides (<0)).

The results of the PECs calculations for 'hydrolysed proteins' are presented in Table 9.1.3-4.

Table 9.1.3-4: PEC_{soil} for hydrolysed protein on fruit trees (covering citrus and olive)

PEC_{soil} (mg/kg)		Fruit trees	
		Single application	
		Actual	TWA
Initial		0.541	
Short term	24h	0.528	0.534
	2d	0.516	0.528
	4d	0.493	0.516
Long term	7d	0.460	0.499
	14d	0.391	0.462
	21d	0.333	0.428
	28d	0.283	0.398
	50d	0.170	0.321
	100d	0.054	0.211

CP 9.2 Fate and Behaviour in Water and Sediment

The whole section is not applicable.

CP 9.2.1 Aerobic mineralisation in surface water

CP 9.2.2 Water/sediment study

CP 9.2.3 Irradiated water/sediment study

CP 9.2.4 Estimation of concentrations in groundwater

CP 9.2.4.1 Calculation of concentrations in groundwater

Predicted environmental concentrations in soil (PEC_{GW})

The PEC_{GW} is calculated for the active substance hydrolysed proteins.

Table 9.2.4.1-1: Critical GAP for PEC_{GW} calculations

Crop	Dose rate
Fruit trees, Citrus fruit, Olive trees	Max. 3 applications at a dose rate of 450 g hydrolysed protein/ha, with a minimum interval of 7 days

CP 9.2.4.1.1 Active substance(s) and relevant metabolite(s) (KCP 9.2.4.1)

The PEC_{GW} is calculated for the active substance by means of FOCUS PEARL v4.4.4 and FOCUS PELMO v5.5.3 for the different representative uses. Tables 9.2.4.1-2 and 9.2.4.1-3 show the input parameters that were used in the calculation.

Table 9.2.4.1-2: Input parameters related to application for PEC_{gw} calculations

Crop	Apple (covering fruit and olive trees) Citrus
Application rate (g as/ha)	Hydrolysed protein: 3 x 450
Number of applications/interval (d)	Maximum 3 applications, minimum 7 days interval
Application dates	Worst-case: first application on the 1st of April
Crop interception (%)	Apple: 65% → appl. rate of 3 x 158 g/ha of hydrolysed protein Citrus: 80% → appl. rate of 3 x 90 g/ha of hydrolysed protein
Frequency of application	annual
Models used for calculation	FOCUS PEARL v4.4.4, FOCUS PELMO v5.5.3

Table 9.2.4.1-3: Input parameters related to active substance hydrolysed protein for PEC_{gw} calculations

Compound	Hydrolysed protein	Value in accordance with EU endpoint y/n/ Reference*
Molecular weight (g/mol)	105.1	lowest molecular weight for an amino acid as worst case value
Water solubility (g/L):	1000	FOCUS default
Saturated vapour pressure (Pa):	1 x 10^{-5}	Chosen as non-volatile as a worst-case approach
DT_{50} in soil (d)	30	Preliminary results of the biodegradability test (OECD 301/310) showed that the active substance is readily biodegradable. Default DT_{50} value for readily biodegradable substances with a solid-water partition coefficient in soil (Kpsoil) <100 l/kg, in accordance with ECHA's Guidance on information requirements and Chemical Safety Assessment, Chapter R.16: Environmental exposure assessment (Table R.16-12 for soil)
K_{foc} (mL/g)/K_{fom}	10/5.9	FOCUS default
1/n	1	FOCUS default
Plant uptake factor	0	FOCUS default

Tables 9.2.4.1-4 and 9.2.4.1-5 summarise the results of the PEC_{GW} calculations for 'hydrolysed proteins' with FOCUS PEARL and FOCUS PELMO respectively.

Table 9.2.4.1-4: PEC_{gw} for hydrolysed protein on various crops (with FOCUS PEARL 4.4.4)

Crop	Scenario	80^{th} Percentile PEC_{gw} at 1 m Soil Depth (µg/L)
		Hydrolysed protein
Apple	Châteaudun	35.11
	Hamburg	54.59
	Jokioinen	42.96
	Kremsmünster	25.35
	Okehampton	23.04
	Piacenza	14.57
	Porto	10.32
	Sevilla	25.12
	Thiva	17.45
Citrus	Piacenza	7.89
	Porto	5.36

	Sevilla	5.44
	Thiva	3.18

Table 9.2.4.1-5: PEC$_{gw}$ for hydrolysed protein on various crops (with FOCUS PELMO 5.5.3)

Crop	Scenario	80th Percentile PEC$_{gw}$ at 1 m Soil Depth (µg/L)
		Hydrolysed protein
Apple	Châteaudun	29.03
	Hamburg	23.70
	Jokioinen	31.58
	Kremsmünster	24.38
	Okehampton	24.83
	Piacenza	23.99
	Porto	14.42
	Sevilla	19.75
	Thiva	15.42
Citrus 3 x 450 g/ha	Piacenza	8.6
	Porto	5.3
	Sevilla	3.6
	Thiva	3.2

The highest PECgw value for hydrolysed proteins results from the PEARL calculations Hamburg scenario for apple, which amounts to 54.59 µg/L. When the exposure is expressed in nitrogen content, the max PECgw equalizes 8.73 µg/L.

The max PECgw for total nitrogen content exceeds the threshold of 0.1 µg/L. However, this does not cause an unacceptable risk to groundwater, since the max PECgw of 8.73 µg/L is far below the threshold set for nitrogen in the Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. This limit, which equals 22.58 mg/L nitrogen, is almost 1000 times higher than the max total PECgw of 8.73 µg/L.

Moreover, the acceptable risk is confirmed by the fact that the total amount of nitrogen applied per hectare per year is 216 g for the representative product, which is well below the limit set of 77 kg/ha nitrogen, which is the total amount of nitrogen that - according to the Nitrate Directive - can be applied in nitrate vulnerable zones.

CP 9.2.4.2 Additional field tests

CP 9.2.5 Estimation of concentrations in surface water and sediment

Predicted environmental concentrations in soil (PEC_{SW})

Predicted environmental concentrations in soil (PEC_{SED})

The PEC_{SW} is calculated for the active substance 'hydrolysed proteins'.

Table 9.2.5-1: Critical GAP for $PEC_{SW/SE D}$ calculations

Crop	Dose rate
Fruit trees, Citrus fruit, Olive trees	Max. 3 applications at a dose rate of 450 g hydrolysed protein/ha, with a minimum interval of 7 days

CP 9.2.5.1 Active substance(s), relevant metabolite(s) and the formulation (KCP 9.2.5)

The PECsw is calculated for the active substance by means of FOCUS Steps 1-2 for the different crop types. Tables 9.2.5-2 and 9.2.5-3 show the input parameters that were used in the calculations.

Table 9.2.5-2 Input parameters related to $PEC_{SW/SED}$ calculations

Crop type	Pome/stone fruit (late application) Citrus Olives
Application rate (g as/ha)	Hydrolysed protein: 3 x 450
Number of applications/interval (d)	Max. 3 application with a minimum interval of 7 days
Application window	Mar-May / Jun-Sep Southern Europe
Application method	Spraying
Interception class (relevant for STEP 2)	Average cover
Models used for calculation	FOCUS STEP 1-2

Table 9.2.5-3 Input parameters related to hydrolysed protein for $PEC_{SW/SED}$ calculations

Compound	**Hydrolysed protein**	**Value in accordance to EU endpoint / Reference**
Water solubility (g/L)	1000	FOCUS default
Diffusion coefficient in water (m²/d)	4.3×10^{-5}	FOCUS default
Diffusion coefficient in air (m²/d)	0.43	FOCUS default
K_{foc} (mL/g)	10 (for PECsw)/10000 (for PECsed)	FOCUS defaults
Freundlich Exponent 1/n	1	FOCUS default
Plant Uptake	0	FOCUS default
$DT_{50,soil}$ (d)	30	Preliminary results of the biodegradability test (OECD 301/310) showed that the active substance is readily biodegradable. Default DT_{50} value for readily biodegradable substances with a solid-water partition coefficient in soil (Kpsoil) <100 l/kg, in accordance with ECHA's Guidance on information requirements and Chemical Safety Assessment, Chapter R.16: Environmental exposure assessment (Table R.16-12 for soil).
$DT_{50,water}$ (d)	15	Default DT_{50} value for readily biodegradable substances, in accordance with ECHA's Guidance on information requirements and Chemical Safety Assessment, Chapter R.16: Environmental exposure assessment (Table R.16-11 for surface water).
$DT_{50,sed}$ (d)	1000	FOCUS default
$DT_{50,whole\ system}$ (d)	1000	FOCUS default

Predicted concentrations in surface water and sediment were determined using FOCUS Steps 1-2 calculations. The results are shown in Table 9.2.5-4.

Table 9.2.5-4 FOCUS Step 1 and 2 PEC_{sw} and PEC_{sed} for hydrolysed protein following three applications (3 x 450 g/ha) to pome fruits, citrus and olive trees

Pome/stone fruit					
Scenario **FOCUS**	**Waterbody**	**Max PEC_{sw} (µg/L)**	**Dominant entry route**	**21 d- $PEC_{sw,twa}$ (µg/L)**	**Max PEC_{sed} (µg/kg)**
Step 1	---	514.84	---	510.21	3630
Step 2	---		---		

Southern Europe	March-May	144.07		73.30	909.92
	June-Sept	93.22		59.89	762.65
Citrus					
Scenario FOCUS	**Waterbody**	**Max PEC$_{sw}$ (µg/L)**	**Dominant entry route**	**21 d- PEC$_{sw,twa}$ (µg/L)**	**Max PEC$_{sed}$ (µg/kg)**
Step 1	---	514.84	---	510.21	3630
Step 2	---		---		
Southern Europe	March-May	51.53		33.07	517.20
	June-Sept	51.53		33.07	517.20
Olives					
Scenario FOCUS	**Waterbody**	**Max PEC$_{sw}$ (µg/L)**	**Dominant entry route**	**21 d- PEC$_{sw,twa}$ (µg/L)**	**Max PEC$_{sed}$ (µg/kg)**
Step 1	---	514.84	---	510.21	3630
Step 2	---		---		
Southern Europe	March-May	72.38		46.48	615.38
	June-Sept	61.96		39.78	541.75

The max PECsw for pome fruit to be used in the risk assessment is 144.07 µg/L, resulting from Step 2 scenario March-May, which amounts to 23.05 µg/L expressed in total nitrogen.

The max PECsw for citrus to be used in the risk assessment is 51.53 µg/L, resulting from Step 2 scenario March-May, which amounts to 8.24 µg/L expressed in total nitrogen.

The max PECsw for olives is 72.38 µg/L, resulting from Step 2 scenario March-May, which amounts to 11.58 µg/L expressed in total nitrogen.

CP 9.3 Fate and Behaviour in Air

The fate and behaviour in air of Hydrolysed Proteins was not evaluated during the Annex I Inclusion, but justification was provided and found acceptable. No additional studies have been performed.

The ‘hydrolysed proteins’ are a complex mixture, mainly containing amino acids and peptides. Vapour pressure is a physico-chemical parameter that cannot be measured for mixtures. Hence, the data requirement for a study on vapour pressure is waived, based on the fact that such a study is not technically feasible. A reasoned case is presented below to describe the volatility based on the composition of the respective sources of ‘hydrolysed proteins’, e.g. amino acids.

A relevant parameter to describe the potential volatilization of the components from the technical active substance (aqueous solution) is Henry’s Law Constant, as this describes the partitioning between the aqueous and the gas phase.

For amino acids, Henry’s Law Constants at 25°C are reported to be between 10^7 and 10^{13} M/atm (ref. 1), corresponding to 10^2 to 10^8 mol/(m^3.Pa). Expressed in its dimensionless form (i.e. as the ratio of concentration in the aqueous phase to the concentration in the gas phase) this gives $2.5x10^5$ to $2.5x\ 10^{11}$.

Taken together, the main components of the hydrolysed proteins mixtures have a ratio of concentration in the aqueous phase to concentration in the gas phase of $>10^5$. Therefore, they can be considered to have a very low potential for volatilisation from water, as well as from moist soil.

In addition, amino acids are very short-lived in air, going by their estimated half-lives in air due to reaction with hydroxyl radicals. According to estimations with AOPWin v. 1.92a (Sept 2010) (EPISuite 4.1; US EPA), the half-life of e.g. arginine at 25°C is 0.946 hours, for leucine 3.034 hours, for glycine 4.578 hours.

It is noted that the putative mode of action of the ‘hydrolysed proteins’ is to attract insects, following spraying of the ‘hydrolysed proteins’ formulations and subsequent evolvement of small-molecule volatile compounds with organoleptic qualities attracting flies (e.g. NH_3). These biologically active compounds only emerge on-site in minor quantities.

[1] Compilation of Henry’s Law Constants for Inorganic and Organic Species of Potential Importance in Environmental Chemistry; R. Sander; Air Chemistry Department Max-Planck Institute of Chemistry, Mainz, Germany. Version 3 (February 17, 1999).

CP 9.3.1 Route and rate of degradation in air and transport via air

Predicted environmental concentrations from airborne transport

CP 9.4 Estimation of Concentrations for Other Routes of Exposure

Not applicable