BIOCEBO/BIO

DOCUMENT M-CP, Section 7

TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCT

Version history[1]

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-AnnexIII
2018-01-09	Toxicological studies were performed in 2013 to adapt BIOCEBO dossier to Uniform Principles. Summary results are incorporated for the following toxicity tests: acute LD50 oral (rat), acute LD50 dermal (rat), acute LC50 inhalation (rat), skin irritation (rabbit), eye irritation (rabbit) and sensitization of the skin [Buehler test (guinea pig)].	DOCUMENT M-CP, Section 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

Table of Contents

CP 7 TOXICOLOGICAL STUDIES ON THE PLANT PROTECTION PRODUCTS

Introduction

This document reviews the toxicological studies for the product BIOCEBO containing the active substance Hydrolysed Proteins, which was included into Annex I of Directive 91/414/EEC. The Annex I Inclusion Directive for Hydrolysed Proteins (**2009/153/EC)** provides specific provisions under Part B which need to be considered by the applicant in the preparation of their submission and by the MS prior to granting an authorisation. A full risk assessment according to Uniform Principles is provided which demonstrates that the product is safe for operators, workers and bystanders.
Where appropriate this document refers to the conclusions of the EU review of the Hydrolysed Proteins. The SANCO review report for Hydrolysed Proteins (SANCO/2615/08 rev 3 – 03/10/2009) is considered to provide the relevant information or a reference to where such information can be found.

BIOCEBO's formulation, based on Hydrolysed Proteins, is devoid of toxicity. We have performed the acute toxicity studies in a product containing a higher concentration of hydrolysed proteins (FLYRAL, which contains 36% w/v of hydrolysed proteins). BIOCEBO and FLYRAL are both products property of BIOIBERICA, S.A. which is the company manufacturing both pest protection products. Both products contain Hydrolysed proteins as its main and only ingredient, but the difference between both of them is the concentration in hydrolysed proteins (BIOCEBO contains 30% w/v of Hydrolysed proteins and FLYRAL 36% w/v). Being FLYRAL more concentrated in Hydrolysed proteins, it has been considered as the worst-case scenario, complying with the Good Laboratory Practices and avoiding duplication in toxicity studies with animals. Therefore, results of the toxicity studies of FLYRAL are also applicable for BIOCEBO as this last is less concentrated, and thus, it is expected to be safer than FLYRAL.

The toxicology area evaluation of the Registration Reports from Spanish Authorities concluded that the only potential risk posed by these hydrolysed proteins is their possible microbial pathogen contamination by BSE risk, and this risk is ruled out by compliance with Commission Regulation (EU) No. 142/2011, implementing Regulation (EC) Nº 1069/2009 laying down health rules regarding animal-by products. Moreover, concluded that BIOCEBO was not classified for any of the toxicity tests performed.

Acute Toxicity

Summary of acute toxicity

The following tests were performed on FLYRAL: acute LD_{50} oral (rat), acute LD_{50} dermal (rat), acute LC_{50} inhalation (rat), skin irritation (rabbit), eye irritation (rabbit) and sensitization of the skin [Buehler test (guinea pig)]. The results are summarised in table below.

Acute toxicological data obtained with FLYRAL

Parameter [Reference]	**Species**	**Result mg/kg or mg/m³ or effect**	**Classification**
Acute LD_{50} oral [ES/12/002]	rat	2000 mg/Kg < DL50 ≤ 5000 mg/Kg	Not toxic
Acute LD_{50} dermal [ES/12/003]	rat	> 2000 mg /kg	Not toxic
Acute LC_{50} inhalation [41200164]	rat	> 5.11 mg/L for 4 hours	Not toxic
Skin irritation [ES/12/004]	rabbit	Effect not detected	No effect
Eye irritation [ES/12/005]	rabbit	Effect not detected	No effect
Sensitization of the skin [ES/12/001]	guinea pig	Effect not detected	No effect

FLYRAL containing 307 g/kg (360 g/L) of Hydrolysed Proteins has a very low toxicity in respect to acute oral and dermal toxicity, and is neither irritating to the rabbit eye and the rabbit skin nor skin sensitizer. Taking into account that, BIOCEBO is less concentrated in hydrolysed proteins than FLYRAL and that all submitted data and the labelling of the active substance as nontoxic, BIOCEBO should not be labelled with risk or caution phrases.

According to the Directive 2001/59/EC, there is not a proposed toxicological classification for BIOCEBO. The product can be deemed as nontoxic.

CP 7.1.1 Oral toxicity

Report:	**IIIA1 7.1.1/01, Núria Àlvarez, 2012**
Title:	Estudio de toxicidad aguda oral de FLYRAL en rata. Study of acute oral toxicity of FLYRAL in rat.
Document No:	ES/12/002
Guidelines:	OECD Guideline for testing of chemicals 432. Acute Oral Toxicity – Acute Toxic. Class Method (Adopted: 17.12.2001).
GLP	Yes

Material and Methods:

FLYRAL (batch No. 11/0001) is a dark brown liquid containing the Hydrolysed. A single administration of 2,000 mg/kg of the undiluted test substance was administered by gavage to 6 Sprague Dawley (SD) female rats divided in two groups of three rats each. The administration volume was 0.17 mL/100 g bw.

Acute oral toxicity in rats of FLYRAL

Dose (mg/kg)	**Toxicological results***	**Duration of signs**	**Time of death**	**LD_{50} (mg/kg) (14 days)**
Group 1				
2,000	0/0/3	-	-	2000 mg/Kg < LD50 ≤ 5000 mg/Kg
Group 2				
2,000	0/0/3	-	-	2000 mg/Kg < LD50 ≤ 5000 mg/Kg

* Number of animals which died / number of animals with clinical signs / number of animals used

Findings:

- No clinical signs and no deaths were observed during the study.
- The body weight variations were considered as normal in the rat strain used for the study.
- Macroscopic examination revealed no significant alterations in any of the animals.

Conclusion/endpoint:

Under the experimental conditions, according to the above-mentioned method, and due to that no deaths were observed among the animals during the whole study, it is considered that the product FLYRAL has an oral LD50 in rat of: 2000 mg/Kg < DL50 ≤ 5000 mg/Kg.
Therefore, product BIOCEBO which has the same active ingredient, hydrolyzed proteins, but it is less concentration, also has an oral LD50 in rat of: 2000 mg/Kg < DL50 ≤ 5000 mg/Kg.

CP 7.1.2 Dermal toxicity

Report:	**IIIA1 7.1.2/01, Núria Àlvarez, 2012**
Title:	Estudio de toxicidad aguda dérmica de FLYRAL en rata. Study of dermal acute toxicity of FLYRAL in rat.
Document No:	ES/12/003
Guidelines:	OECD Guideline for testing of chemicals 402. Acute Dermal Toxicity (24 Feb 1987).
GLP	Yes

Material and Methods:

FLYRAL (batch No. 11/0001) is a dark brown liquid containing the Hydrolysed Proteins. The sample was applied topically to an area equivalent to approximately 10% of the total body surface of the animal. A single administration of 2,000 mg/kg of the undiluted test substance was administered to 10 Sprague Dawley (SD) rats (5 male/5 female). The administration volume was 0.17 mL/100 g bw.

Acute dermal toxicity in rats of FLYRAL

Dose (mg/kg)	**Toxicological results***	**Duration of signs**	**Time of death**	**LD_{50} (mg/kg) (14 days)**
male rats				
2000	0/0/5	-	-	> 2000
female rats				
2000	0/0/5	-	-	> 2000

* Number of animals which died/number of animals with clinical signs/number of animals used

Findings:

- No clinical signs and no deaths were observed during the study.
- The body weight variations were considered as normal in the rat strain used for the study.
- Macroscopic examination revealed no significant alterations in any of the animals.

Conclusion/endpoint:

Under the experimental conditions, according to the above-mentioned method, and due to that no deaths were observed among the 10 animals treated during the whole study, it is considered that the product FLYRAL has a dermal LD50 in rat of: > 2000 mg/Kg.

Therefore, product BIOCEBO which has the same active ingredient, hydrolyzed proteins, but it is less concentration, has also a dermal LD50 in rat of: > 2000 mg/Kg.

CP 7.1.3 Inhalation toxicity

Report:	**IIIA1 7.1.3/01, D.R. Griffiths, 2012**
Title:	FLYRAL: Acute inhalation toxicity (nose only) study in the rat
Document No:	Harlan Laboratories Study 41200164
Guidelines:	OECD Guidelines for testing of chemicals (2009) nº 436 – Acute inhalation toxicity – Acute Toxic Class Method.
GLP	Yes

Material and Methods:

FLYRAL (batch No. 11/0001) is a dark brown liquid containing the Hydrolysed Proteins. A group of 6 RccHan^{TM} : WIST strain rats (3 males and 3 females) were exposed to an aerosol atmosphere for four hours using a nose-only exposure system, following a 14 day observation period. The mean atmosphere concentration achieved was of 5.11 mg/L air (5,100 mg/m^3).

Acute inhalation toxicity in rats of FLYRAL

Dose (mg/L)	**Toxicological results***	**Duration of signs**	**Time of death**	**LC_{50} (mg/L) (14 days)**
male rats				
5.11	0/0/3	-	-	> 5.11
female rats				
5.11	0/0/3	-	-	> 5.11

* Number of animals which died / number of animals with clinical signs / number of animals used

Findings:

- All animals survived. Common abnormalities noted during the study.
- Reasonable bodyweight gains were noted in all animals during the recovery period.
- No macroscopic abnormalities were detected amongst animals at necropsy.

Conclusion/endpoint:

No deaths occurred, therefore, there was no indication of relevant sex-related differences in toxicity of the test item in a group of six rats exposed to a mean achieved atmosphere concentration of 5.11 mg/L for four hours. It was therefore considered that the acute inhalation median lethal concentration (4hr LC50) of FLYRAL in the strain rats used was greater than 5.11 mg/L.

Considering FLYRAL as a worst-case scenario, it is also expected BIOCEBO to be safe and has a low lethal concentration.

CP 7.1.4 Skin irritation

Report:	**IIIA1 7.1.4/01, Núria Àlvarez, 2012**
Title:	Estudio de irritación/corrosion dérmica FLYRAL en conejo. Study of skin irritation of FLYRAL in rabbit.
Document No:	ES/12/004
Guidelines:	OECD Guideline for testing of chemicals 404 Acute Dermal Irritation/Corrosion (Adopted: 24th April 2002)
GLP	Yes

Material and Methods:

FLYRAL (batch No. 11/0001) is a dark brown liquid containing the Hydrolysed Proteins. To three male New Zealand White rabbits, a single application of 0.5 mL of the sample was applied to the shaved skin for 4 hours, taking as control the rabbit intact skin. The severity of the dermal irritation or skin corrosion was evaluated 1, 24, 48 and 72 hours after administration.

Skin irritation in rats of FLYRAL

Dose (mg/kg)	**Toxicological results***	**Duration of signs**	**Time of death**	**1 and 2 days**
Treatment group (males)				
0.5 mL	0/0/3	-		No sensitizing effect

* Number of animals which died/number of animals with clinical signs/number of animals used.

Findings:

- No clinical signs observed after 72 hours in the 3 rabbits.
- No skin irritation was detected.
- No risk or safety phrase is required by the product.

Conclusion/endpoint:

Under the experimental conditions, no skin irritation/corrosion effect is produced by FLYRAL in any of the rabbits used for the study.

Therefore, it is neither expected a skin irritation/corrosion for BIOCEBO as it is less concentrated in hydrolysed proteins than FLYRAL.

CP 7.1.5 Eye irritation

Report:	**KIIIA1 7.1.5/01, Núria Àlvarez, 2012**
Title:	Estudio de irrritación/corrosión ocular de FLYRAL en conejo. Study of eye irritation of FLYRAL in rabbits.
Document No:	ES/12/005
Guidelines:	OCDE 405 Acute Eye irritation/Corrosion (Adopted: 24th April 2002)
GLP	Yes

Material and Methods:

FLYRAL (batch No. 11/0001) is a dark brown liquid containing the Hydrolysed Proteins. To three male New Zealand White rabbits, a single application of 0.1 mL of the sample was applied to the left eye, the right one serving as control. The severity of the irritation was observed after 1, 24, 48 and 72 hours after administration.

Eye irritation in rabbits of FLYRAL

Dose (mg/kg)	**Toxicological results***	**Duration of signs**	**Time of death**	**1 and 2 days**
0.1 mL	0/0/3	72 hours	-	No sensitizing effect

* Number of animals which died/number of animals with clinical signs/number of animals used.

Findings:

- One rabbit: important tear drops and coloration observed at periocular area that disappeared in 24 h.

Conclusion/endpoint:

Under the experimental conditions, no eye irriation, neither eye damage is produced by FLYRAL in rabbits. Therefore, it is neither expected eye irritation neither eye damage for BIOCEBO as it is less concentrated in hydrolyzed proteins than FLYRAL.

CP 7.1.6 Skin sensitization

Report:	**KIIIA1 7.1.6/01, Núria Àlvarez, 2010**
Title:	Estudio de sensibilización cutánea de FLYRAL en cobayo. Study of skin sensitisation of FLYRAL in guinea pigs.
Document No:	ES/12/001
Guidelines:	OECD Guideline for testing of chemicals 406. skin sensitisation (17 July 1992).
GLP	Yes

Material and Methods:

FLYRAL (batch No. 11/0001) is a dark brown liquid containing the Hydrolysed Proteins. The maximum allowable concentration for the test was previously determined with 2 animals. Thirty female Dunkin Hartley guinea pigs were submitted to an induction phase (epidermal and intra-dermal). Fifteen days later 20 test animals are exposed to a stimulation dose, to detect any induced hyper-sensibility. A control group (10) was run in parallel. The test substance (alone, diluted with complete Freund adjuvant (carrier), and a blank) was administered intra-dermally and topically 15 days after induction, and effects evaluated after 24 and 48 hours.

Skin sensitization in rabbits of FLYRAL

Dose (mg/kg)	**Toxicological results***	**Duration of signs**	**Time of death**	**After 72 hours**
Treatment group				
a) Carrier-water (1:1) b) Sample c) Sample-carrier-water (1:1)	4/0/20	-	**	No sensitizing effect
Control group				
Carrier-water (1:1) Sample Sample-carrier-water (1:1)	3/0/10	-	***	No sensitizing effect

* Number of animals which died/number of animals with clinical signs/number of animals used.

** Guinea pigs nº 16 and nº 17 died 1 day after induction phase. Nº 18 died 2 days after induction phase. Nº 5 died 14 days after induction phase. All deaths were considered to be not related to study product.

*** Guinea pig nº 26 died 1 day after induction phase. Nº 24 died 7 days after induction phase. Nº 28 died 15 days after induction phase. All deaths were considered to be not related to study product.

Findings:

- Animals from both groups died for causes not related to the test or to the product.
- Less than 30% of the animals (11.1%) showed sensitising effect compared with the control group.
- No risk and safety phrases are required in the label of the product.

Conclusion/endpoint:

Under the experimental conditions, it is considered that FLYRAL does not produce sensitising to the skin. Therefore, it is also considered that BIOCEBO does not produce sensitising to the skin as is less concentrated in hydrolysed proteins than FLYRAL.

CP 7.1.7 Supplementary studies on the plant protection product

Not required

CP 7.1.8 Supplementary studies for combinations of plant protection product

Not required

CP 7.2 Data on Exposure

CP 7.2.1 Operator exposure

Operator exposure was not evaluated as part of the EU review of Hydrolysed Proteins for the submitted rate/crop. Therefore, all relevant data and risk assessments are provided here and are considered adequate.

Usage information pertinent to operator exposure is summarised in the table above. Since no NOAEL or NOEL values are available, the establishment of an AOEL is not possible and it is not considered necessary.

Summary of critical use patterns (i.e. worst case)

Crop (indoor / field)	Application rate (as/ha)	Spray dilution (L/ha)	Application equipment	Number applications
Field	450 g/ha	1.50 L/ha	Sprayer	3 / season *

These critical use patterns have been defined following evaluation of the GAP (see Appendix 2).

* To be modified according to the instructions of use of the associated/mixed insecticide, the particular variety of the crop and pest pressure level.

Conclusion

The nontoxic characteristics of BIOCEBO, when used under the proposed conditions, give an overall low risk, if any, to the operator. Only the risk inhalation must be considered (see IIIA 7.1.3 acute inhalation toxicity to rats). Accordingly, to the results of this study, the operator exposure risk is negligible.

Risk assessment for operator

CP 7.2.1.1 Estimation of operator exposure

Not applicable

CP 7.2.1.2 Measurement of operator exposure

Not applicable

CP 7.2.2 Bystander and resident exposure

Estimated bystander exposure to Hydrolysed Proteins and % of the AOEL

	A.S. (AOEL = xxxx)
Dermal exposure (mg/kg bw/day)	> 2,000
Inhalation exposure (mg/kg bw/day)	5.11 mg/L air
Total systemic exposure (mg/kg bw/day)	
% of AOEL	

Conclusion

Bystander exposure to BIOCEBO is not to be taken into consideration when using BIOCEBO under proper use conditions. Also, according to the toxicity studies results, no toxicity concerns should be expected from the use of the product under the proper conditions of use.

The Final Report on Hydrolysed proteins of 1st June 2012, approved by the Standing Plant Protection Committee (SANCO/2615/08*-rev.04), did not set out the relevant toxicological parameters for hydrolysed proteins risk assessment (IDA, AOEL, oral absorption, ARfD).

Risk assessment for bystander and resident

CP 7.2.2.1 Estimation of bystander and resident exposure

Bystander exposure to BIOCEBO is not to be taken into consideration when using BIOCEBO under proper use conditions. See rationale in point 7.2.2.

CP 7.2.2.2 Measurement of bystander and resident exposure

Not applicable

CP 7.2.3 Worker exposure

Risk assessment for worker

Estimated worker exposure to Hydrolysed Proteins

		Exposure parameter	
Active substance	**AOEL [mg/kg bw/day]**	**unprotected worker[1]**	
		Absorbed dose [mg/kg bw/day]	% of AOEL
Hydrolysed Proteins	Not available	Not applicable	Not applicable

[1)] Worker wearing shoes, socks, long-sleeved shirt, and long trousers.

Conclusion

Due to the nature of Hydrolysed proteins, it can be concluded that there is no unacceptable risk anticipated for the worker wearing adequate work clothing (but no PPE), when re-entering crops treated with BIOCEBO.

CP 7.2.3.1 Estimation of worker exposure

See rationale in the previous point.

CP 7.2.3.2 Measurement of worker exposure

Not measured

CP 7.3 Dermal Absorption

According to the acute toxicology studies performed [acute percutaneous (dermal) toxicology, skin irritation and skin sensitization], there is no risk associated to the handling of the product. Because of that, no further studies were undertaken.

CP 7.4 Available Toxicological Data Relating to Co-Formulants

Not applicable