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EURL Evaluation Report on the Analytical Methods submitted in connection with the Application for the Authorisation of Feed Additives according to Regulation (EC) No 1831/2003

Dossier related to:	**FAD-2010-0079** **CRL/100163**
Product Name:	***Suilectin***
Active Substance(s):	***Kidney bean lectin (Phaseolus vulgaris)***
Rapporteur Laboratory:	**European Union Reference Laboratory for Feed Additives (EURL-FA)** **Geel, Belgium**
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Report revised by: Date:	**Piotr Robouch (EURL-FA)** **30/06/2011**
Report approved by: Date:	**Christoph von Holst** **01/07/2011**

EXECUTIVE SUMMARY

In the current application authorisation is sought under article 4(1) for *Suilectin* under the category/functional group 4(d) "zootechnical additives"/"other zootechnical additives" (performance enhancer), according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant, *Suilectin* is a natural product in a form of a white to cream fine powder containing standardized *lectins* isolated from red kidney beans (*Phaseolus vulgaris*). The *kidney bean lectin* is a mixture of five phytohaemagglutinin (PHA) isoforms (PHA-E_4, PHA-E_3L, PHA-E_2L_2, PHA-EL_3 and PHA-L_4). Biological activity of the lectin is expressed as haemagglutination activity in Haemagglutination Activity Units (HAU). One haemagglutination unit (HAU) is defined as the amount of material (1 mg/mL) in the last dilution giving 50% agglutination (clumping) of the red blood cells. Therefore, one HAU describes the ability of 1 mg of the tested lectin preparation dissolved in 1 ml of physiological saline (1 mg/ml) to agglutinate at least 50% of the red blood cells. Specifically, authorisation is sought for the use of the *feed additive* for suckling piglets. The *feed additive* is intended to be applied at a dose ranging from 130 to 320 HAU /kg of body weight. It is distributed as a single use bottle with 50 g powder containing 64000 HAU, to be filled up with water prior to use.

For the quantification of activity of *kidney bean lectin* in the *feed additive*, the Applicant proposed a single laboratory validated and further verified haemagglutination assay. The assay is based on the ability of lectins to agglutinate rat erythrocytes. The Applicant performed validation and verification studies, running a series of assays demonstrating reproducibility of the method by always obtaining haemagglutination of 50% erythrocytes at the fourth dilution. Furthermore, the Applicant reported a limit of detection (LOD) of 0.64 HAU/mL. The EURL considers haemagglutination assay suitable for official control for the quantification of *kidney bean lectin* in the *feed additive*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

KEYWORDS
suilectin, kidney bean lectin, Phaseolus vulgaris, zootechnical additive, other zootechnical additives, performance enhancer, pigs, suckling piglets.

1. BACKGROUND

In the current application authorisation is sought under article 4(1) (authorization of a new feed additive) for *Suilectin* under the category/functional group 4(d) "zootechnical additives"/"other zootechnical additives" (performance enhancer) [1,2], according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant, *Suilectin* is a product containing standardized *lectins* isolated from red kidney beans (*Phaseolus vulgaris*) [2,3]. The *kidney bean lectin* is a mixture of five phytohaemagglutinin (PHA) isoforms (PHA-E_4, PHA-E_3L, PHA-E_2L_2, PHA-EL_3 and PHA-L_4), composed of two molecular species: leucoagglutinin (PHA-L) and erythroagglutinin (PHA-E). *Suilectin* is a natural product in a form of a white to cream fine powder mixed with maltodextrin as a carrier and contains 12.5 – 25% pure PHA [3]. Biological activity of the lectin is expressed as haemagglutination activity in Haemagglutination Activity Units (HAU). One haemagglutination unit (HAU) is defined as the amount of material (1 mg/mL) in the last dilution giving 50% agglutination (clumping) of the red blood cells [4]. Therefore, one HAU describes the ability of 1 mg of the tested lectin preparation dissolved in 1 ml of physiological saline (1 mg/ml) to agglutinate at least 50% of the red blood cells. Specifically, authorisation is sought for the use of the *feed additive* for suckling piglets [2]. The *feed additive* is intended to be applied at a dose ranging from 130 to 320 HAU /kg of body weight [2,3]. It is distributed as a single use bottle with 50 g powder containing 64000 HAU, which is filled up with water prior to use [2].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the European Union Reference Laboratory concerning applications for authorisations of feed additives, the EURL is requested to submit a full evaluation report to the European Food Safety Authority for each application or group of applications. For this particular dossier, the methods of analysis submitted in connection with *Suilectin*, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, mycotoxins, and dioxins) are available from the respective European Union Reference Laboratories [5].

Description of the analytical methods for the quantification of the active substance in feed additive, premixtures and feedingstuffs

For the quantification of activity of *kidney bean lectin* in the *feed additive*, the Applicant proposed a single laboratory validated and further verified haemagglutination assay [6], based on modified Pusztai and Watt's method [7]. The assay is based on the ability of *lectins* to agglutinate rat erythrocytes. The sample (200 mg of *Suilectin* with a known activity of 128 HAU/100 mg) is dissolved in 8 mL of physiological saline (0.9% NaCl) to a final concentration of 25 mg/mL and a series of double-fold increasing diluted solutions ($1/2^i$ ($i = 1, 7$) dilution) is prepared. Appropriate test solution dilutions (100 µl) are mixed with 100 µl 2% erythrocyte suspension and 100 µl physiological saline. After mixing, 50 µl is placed on a microscope slide and incubated in humid chamber at room temperature for 30 to 60 minutes. The remaining reaction solution is incubated in test tube at least for 60 minutes. After the incubation, the mixture is mixed and 50 µl collected and placed on microscope slide. A negative control (200 µl of physiological saline and 100 µl of 2% erythrocyte solution) is performed parallel to the test. The reading of the test is performed under 250x magnification in a light microscope, checking for presence of haemagglutination. The number of HAU for 1 mg of tested lectin preparation is defined by the reverse concentration of the solution (expressed in mg/ml) in the consecutive dilution, in which haemagglutination of at least 50% of erythrocytes is still being observed. The Applicant performed validation [8] and verification studies [9 – 12], running a series of assays demonstrating reproducibility of the method by always obtaining haemagglutination of 50% erythrocytes at the fourth dilution (1/32, $i = 4$). Furthermore, the Applicant reported a limit of detection (LOD) of 0.64 HAU/mL. The EURL considers haemagglutination assay suitable for official control for the quantification of *kidney bean lectin* in the *feed additive*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control the single laboratory validated and further verified haemagglutination assay for the quantification of *lectin* in *feed additive*.

Recommended text for the register entry (analytical method)

For the quantification of the *kidney bean lectin* in the *feed additive*:

- haemagglutination assay

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *Suilectin* have been sent to the European Union Reference Laboratory for Feed Additives. The dossier has been made available to the EURL by EFSA.

6. REFERENCES

[1] *Application, Reference SANCO/D/2 Forw. Appl. 1831/060-2010

[2] *Application, Proposal for Register Entry – Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive; Methods of analysis

[4] *Technical dossier, Section II - Protocol for verification studies of laboratory validated methods ANNEX A

[5] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[6] *Technical dossier, Section II – Annex_II_6.B. Procedure for lectin activity quantification 01.09.2008

[7] *Technical dossier, Section II, Annex_II_6.A. Pusztai et al 1979

[8] *Technical dossier, Section II, Annex_II_6.C. Serol method validation report

[9] *Technical dossier, Section II, Annexes, Protocol for verification studies of laboratory validated methods

[10] *Technical dossier, Section II, Annexes, Protocol for verification studies of laboratory validated methods ANNEX A

[11] *Technical dossier, Section II, Annexes, Protocol for verification studies of laboratory validated methods ANNEX B

[12] *Technical dossier, Section II, Annex_II_6.D. Verification report of biological activity of lectins

* Refers to Dossier No. FAD-2010-0251

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was European Union Reference Laboratory for Feed Additives, IRMM, Geel, Belgium. This report is in accordance with the opinion of the consortium of National Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)