Addendum 2 to CRL report (No. D08/FSQ/AMJ/CVH/(2005) D 21715) on the dossier related to EFSA-Q-2004-173 (Formi™ LHS)

Additional conclusion regarding the suitability of the analytical method for official control purposes:
The analytical method for the determination of the active substance in feedingstuff is, however, considered suitable for official control purposes, if the analysis aims at the determination of the active substance in feedingstuffs based on the measurement of the *total* formate content, which means regardless of its origin.

Addendum 1 to CRL report (No. D08/FSQ/AMJ/CVH/(2005) D 21715) on the dossier related to EFSA-Q-2004-173 (Formi™ LHS)

Background
The dossier related to EFSA-Q-2004-173 is on the feed additive Formi™ LHS which contains the active substance potassium diformate. The proposed registry entry contains minimum and maximum limits for the active substance in feed.
For the detection of the active substance in feedingstuffs the applicant proposed an ion chromatography method with conductivity detection which measures the content of the anion formate in the sample. Based on the measured formate content the concentration of potassium diformate in the sample is calculated.
The CRL concludes that this method is not suitable for official control purposes, since it is not able to discriminate if the measured formate in the sample originates from Formi™ LHS or from other formate sources added to the feedingstuff.

Reason for addendum
The report has been finalised and sent to EFSA on 5 September 2005. In between intensive discussions between the CRL, EFSA, European Commission DG Health and Consumer Protection and the National Reference Laboratories (NRLs) took place about the precise objectives of the CRL evaluation reports. One important aspect of these discussions was the clarification of the requirements for official control, focusing on the proposed conditions for authorisation of the feed additive concerned. To take these aspects into account, the CRL decided to add the following conclusion to the CRL report for this dossier.

Additional conclusion regarding the suitability of the analytical method for official control purposes:
The analytical method for the determination of the active substance in feedingstuff is, however, considered suitable for official control purposes, if the analysis aims at the determination the active substance in feedingstuffs based on the measurement of the *total* formate content, which means regardless of its origin.

CRL Evaluation Report on the Analytical Methods submitted in connection with Section 2.5 (Control Methods) of the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier No.:	FAD-04-004
Name of Additive:	Formi™ LHS
Active Subtance(s):	Potassium diformate
Rapporteur Laboratory:	The Danish Plant Directorate, Lyngby, Denmark.
Report prepared by:	A. Plöger (The Danish Plant Directorate, Lyngby, Denmark)
Report checked by:	A. M. Jensen (CRL-FAA)
Date:	31/08/2005
Report approved by:	C. von Holst (CRL-FAA)
Date:	02/09/2005

1. EXECUTIVE SUMMARY

The objective of this report is to evaluate the analytical methods related to the dossier "FAD No. 04-004" regarding Formi^{TM} LHS. Formi^{TM} LHS is a dry product containing minimum 98% of potassium diformate (CAS no.: 20642-05-1) as the active substance, and the additive is intended for use as a growth promoter for pigs.

The applicant proposes classification of this feed additive in the category "zootechnical additive" and in the functional group "gut flora stabilisers". The application is for approval for 10 years for piglets and pigs for fattening in the target range 6000-18000 mg/kg feedingstuff and 6000-12000 mg/kg feedingstuff, respectively.

All relevant information is given in the dossier, which also contains enclosures and an expert report.

For determining the active substance in the additive, total formate analysis is based on oxidation followed by titration. The result is controlled by the analysis of formic acid and potassium. Formic acid is determined by acid-base titration. The applicant proposes to analyse potassium by gravimetry, atomic absorption spectrometry (AAS) or inductively coupled plasma-atomic emission spectrometry (ICP-AES). In the view of the Community Reference Laboratory, these methods are suitable for the purpose as routine control methods.

An ion chromatography method with conductivity detection is proposed for analysis of Formi^{TM} LHS in feedingstuffs and premixtures. The content of Formi^{TM} LHS is calculated from the potassium diformate content which in turn is calculated from the formate content. The sample is extracted with a mobile phase. After dilution and filtration, the samples are analysed with ion chromatography and conductivity detection. This method has been validated for feedingstuffs. By spiking about 1500 mg/kg formate in pig feed and fish meal (n = 3), 95% recovery and 5% relative standard deviation was found. The limit of detection is 0.6 mg/kg feedingstuff. The limit of quantification is 2 mg/kg feedingstuff. The spiking in the validation of the method of analysis of feedingstuffs is performed at a lower level of formate - 1500 mg/kg feedingstuff – than the target range 6000-18000 mg/kg in feedingstuffs. This is not considered a serious problem, because spiking with a higher level presumably would improve the performance characteristics. The ion chromatographic method, which was submitted by the applicant for analysis of feedingstuff and of target tissues, is sufficiently validated and is considered suitable for routine control. It should be noted that the method for analysis of Formi^{TM} LHS in feedingstuffs is not able to discriminate if the formic acid in the

sample originates from Formi^{TM} LHS or other formic acid sources added to the feedingstuff (acidifiers or preservatives). Therefore, this method of analysis of formic acid in feedingstuff can not be used for verifying the maximum level of Formi^{TM} LHS in feedingstuff, when other sources of formic acid are present, and consequently, it is not considered appropriate for official control purposes. However, to our knowledge, no other routine analytical method would be able to discriminate the source of formic acid in feedingstuff.

While the method for analysis of Formi^{TM} LHS in premixtures has not been validated, this is not considered a serious problem, since the target inclusion levels for the additive in feedingstuff are quite high (in the percent range), and therefore comparable to the levels typically applied in premixtures.

The dossier contains information on the specification for contents of arsenic, cadmium, lead and mercury and the corresponding submitted methods of analysis (ICP-AES or AAS) have limits of quantification (0.02 mg/kg, 1 mg/kg, 2 mg/kg and 0.0003 mg/kg, respectively) well below the specifications . For other inorganics impurities in Formi^{TM} LHS, the methods of analysis (ICP-AES or AAS) are given with the corresponding limits of quantification. The anticaking agent silica, which is in the product at maximum 0.5%, can be analysed with ICP-AES.

Further testing or validation is not considered necessary.

2. KEYWORDS

Potassium diformate, formic acid, ion chromatography, Formi^{TM} LHS

3. TABLE OF CONTENTS

4. BACKGROUND

The name of the additive is Formi^{TM} LHS. This product contains minimum 98.0 % (w/w) potassium diformate, maximum 0.5% (w/w) water and maximum 1.5% (w/w) anticaking agent (e.g. silicate).

Formi^{TM} LHS is used as growth promoter in the feedingstuff for pigs. The additive has been provisionally authorised at Community level as a growth promoter for weaned piglets and pigs for fattening in the target range 6000-18000 mg/kg feedingstuff and 6000-12000 mg/kg feedingstuff, respectively.

The applicant proposes classification of this feedingstuff additive in the category zootechnical additive and in the functional group “gut flora stabilisers”. This application is for approval for 10 years for piglets and pigs for fattening in the target range 6000-18000 mg/kg feedingstuff and 6000-12000 mg/kg feedingstuff, respectively.

All relevant information is given in the dossier, which is structured in the main part of the dossier (00_Section_II_Chem_Dossier_FormiTMLHS_May2004.doc) with enclosures and an expert report.

5. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005 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 tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority for each application. For this particular dossier, the suitability of the control methods and validation studies submitted in connection with Formi^{TM} LHS (FAD-04-004) were evaluated.

6. EVALUATION

The numbering system under this point refers to that of the Section 2.5 (Control Methods) of the Annex of Commission Directive 2001/79/EC.

For those methods for which validation was carried out these were perforemed in-house (see below).

Description of the methods used for the determination of criteria related to the identity and characterisation of the additive; methods of control

(Cf. the requirements listed in point 2.5.1. of the Annex)

Qualitative and quantitative composition (active substance, other components, impurities, batch-to-batch variation)

For quantitative analysis of potassium diformate method HRE-APA-A55E, provided by the applicant, is used (enclosure 25). By using this technique, potassium diformate is calculated from the analysis of total formate (HRE-APP-A49E), and checked by the analysis of formic acid (HRE-APP-A59E) and potassium (HRE-ANA-A83)..

Total formate analysis is based on oxidation with potassium permanganate followed by iodometric titration. (enclosure 28).

Formic acid is determined by acid-base titration. Potassium can be analysed by gravimetry.

The batch-to batch variation has been investigated for potassium diformate, silicate, water, particle size, bulk density and appearance. The product passed the specification for all the analysed batches and the analytical methods are considered suitable. (*Cf.* the requirements listed in point 2.1.3. of the Annex.)

The determination of the physical properties of the additive

Information is given for the particle size distribution, flow characteristics and bulk density together with the concerning methods of analysis. Methods for analysing rheological characterisation is given, too and are considered appropriate. *(Cf*. the requirements listed in point 2.1.4. of the Annex).

Identification and quantification of occurring chemical impurities and toxic substances

The dossier contains informations on the specification for contents of arsenic, cadmium, lead and mercury and the corresponding methods of analysis with limits of quantification (0.02 mg/kg, 1 mg/kg, 2 mg/kg and 0.0003 mg/kg, respectively) well below the specifications - 12 mg/kg, 15 mg/kg, 40 mg/kg and 0.5 mg/kg, respectively (ICP-AES or AAS).

For other inorganics impurities in Formi^{TM} LHS, the methods of analysis are given with the corresponding limits of quantification (ICP-AES or AAS).

Water is analysed with a modification with the Karl Fischer method (enclosure 26).

The anticaking agent silica, which is in the product in max 0.5%, can be analysed with ICP-AES (enclosure 2). The analytical methods are considered suitable. *(Cf*. the requirements listed in point 2.2.3. of the Annex).

Physical properties

An X-ray diffraction diagram for Formi^{TM} LHS is produced by the applicant and is compared to library spectra (enclosure 23). The library identification spectrum for potassium formate is mentioned, it has the number 14-793. This spectrum appears in enclosure 10.

Enclosure 13 gives information of the melting point and the method of determination. Boiling point is analysed according to enclosure 41.

Dissociation constant of the diformate is analysed according to enclosure 39. The solubilities in water and organic solvents are given with the used methods of determination.

Bulk density is given with the method of determination.

Methods for analysing vapour pressures are given (enclosures 42 and 43) without values for vapour pressure of the compound.

The critical relative humidity is given without a method for analysing it.

The analytical methods are considered suitable. *(Cf*. the requirements listed in point 2.2.4. of the Annex.)

Stability of each formulation

The applicant performed studies according to standardised procedures including accelerated test (enclosure 9). The methods used are the same as those mentioned under "*Qualitative and quantitative composition (active substance, other components, impurities, batch-to-batch variation)"*. It was concluded that the additive can be regarded as stable for 6 months under an accelerated stability test. Method HRE-APA-A55 (Potassium diformate) was used based on determination of total formate formic acid and potassium

Up to two years stability studies have been performed. Studies for stability versus moisture and temperature have been performed.

Stability versus moisture and temperature has been analysed with the methods mentioned in enclosures 44 (redox-titration), 45 (acid-base titration) and 36 (weighing of absorption of water). Validation data are not present for any of these 3 methods.

The stability studies show that Formi^{TM} LHS could be stored for up to 12 months.

The methods applied are considered suitable *(Cf.* the requirements listed in point 2.3.1. of the Annex).

Stability in feedingstuffs and premixtures

The stability of Formi^{TM} LHS has been investigated in feeding stuffs and premixtures.

For premixtures, the additive will remain almost unchanged after 11 months storage at room temperature. The analytical method is HRE-NIT A33E (an ion chromatographic method). The recommended storage of premixtures is 6 months.

For feedingstuffs, production trials showed that the additive was lost in amounts up to 8.2%. The analytical method is unknown, and therefore the CRL cannot elaborate of the suitability of this analytical method for this purpose.

Storage of feedingstuffs at ambient temperatures for 6 months showed no loss of Formi^{TM} LHS. Shelf life recommendation is three months for feedingstuffs.

(Cf. the requirements listed in point 2.3.2. of the Annex.)

<u>Description of the qualitative and quantitative analytical methods for routine control of the active substance in premixtures and feedingstuff</u>

The method: "Determination of Formi^{TM} LHS content (K-diformate/formate) in feedstuffs and premixes", (enclosure CRC-ANA-A190.pdf) in "Additional documents" is an ion

chromatography method with conductivity detection. This method has been validated (enclosure ValidationReport_0055_001.pdf) in "Additional documents".

The sample is extracted with mobile phase, 5 mM sodium tetraborate at pH approximately 11.5. After dilution and filtration, the samples is analysed with ion chromatography and conductivity detection. Sodium formate is used for calibration. The content of Formi^{TM} LHS is calculated from the K-diformate content which in turn is calculated from the formate content.

Linearity for the calibration curve is 0.2-12 ug formate/g solution. The correlation coefficient is 0.99. The correlation coefficient indicates an acceptable linearity.

Principles from pharmaceutical guidelines, PharmComm and ICH-guideline have been used for validation of the method.

By spiking about 1500 mg/kg of formate in pig feed, fish feed and fish meal (n = 3): 95% recovery and 5% relative standard deviation was found.

LOD = 0.6 mg/kg in feedingstuff . LOQ = 2 mg/kg in feedingstuff . All performance characteristics are considered acceptable for routine control.

While the method for analysis of Formi^{TM} LHS in premixtures has not been validated, this is not considered a serious problem, since the target inclusion levels for the additive in feedingstuff are quite high (in the percent range), and therefore not far from the levels typically applied in premixtures.

In addition the applicant proposes the enzymatic method "Formic Acid" UV-method from Boehringer Mannheim which in our view can be used for determination of Formi^{TM} LHS in premixtures and feedingstuffs.

(Cf. the requirements listed in point 2.5.2. of the Annex.)

<u>Description of the qualitative and quantitative analytical methods for determining the marker residue(s) of the active substance in target tissues and animal products</u>

The method submitted for determining the additive in feedingstuff also applies for animal tissues. However, formate is endogenously abundant in many animal species including mammals. Its metabolic pathway is well established, and by simple oxidation it is converted into carbon dioxide and water, as explained by the applicant in letter of 20 May 2005. Therefore, and taking into account EFSA´s opinion on Formi^{TM} LHS (see reference) and the report of SCAN of March 2001 on Formi^{TM} LHS, no evaluation is carried out for determining the presence of Formi^{TM} LHS in animal tissues and products.

(Cf. the requirements listed in point 2.5.3. of the Annex)

CHECK LIST

		Y	N	N/A	Comments
1.1	Is/Are the method(s) mentioned in Part I (1. – A. Premixtures) accompanied by information on:				
	- Sampling Method used		X		
	- Percentage Recovery		X		
	- Specificity		X		
	- Accuracy		X		
	- Precision		X		
	- Limit of detection	X			
	- Limit of quantification	X			
	- Validation procedure used		X		See p.9, lines 3-7
1.2	Is/Are the method(s) mentioned in Part I (1. – A. Feedingstuff) accompanied by information on:				
	- Sampling Method used		X		
	- Percentage Recovery	X			
	- Specificity		X		
	- Accuracy	X			
	- Precision	X			
	- Limit of detection	X			
	- Limit of quantification	X			
2.1	Is/Are the method(s) mentioned in Part I (2. – Target tissues) accompanied by information on:				
	- Sampling Method used			X	
.	- Percentage Recovery				
	- Specificity				
	- Accuracy				
	- Precision				
	- Limit of detection				
	- Limit of quantification				
	- Validation procedure used				

2.2	Is/Are the method(s) mentioned in Part I (2. – Animal products) accompanied by information on:			X	
	- Sampling Method used				
	- Percentage Recovery				
	- Specificity				
	- Accuracy				
	- Precision				
	- Limit of detection				
	- Limit of quantification				
	- Validation procedure used				
3.	If the method(s) has/have been devised, consideration has been given to the fact that their limits of quantification must be below the MRLs.	X			MRL is not mentioned.

7. CONCLUSIONS AND RECOMMENDATIONS

For determining the active substance in the additive, total formate analysis is based on oxidation followed by titration. The result is controlled by the analysis of formic acid and potassium. Formic acid is determined by acid-base titration. The applicant proposes to analyse potassium by gravimetry, atomic absorption spectrometry (AAS) or inductively coupled plasma-atomic emission spectrometry (ICP-AES). In the view of the Community Reference Laboratory, these methods are suitable for the purpose as routine control methods.

An ion chromatography method with conductivity detection is proposed for analysis of Formi^{TM} LHS in feedingstuffs and premixtures. The content of Formi^{TM} LHS is calculated from the potassium diformate content which in turn is calculated from the formate content. The sample is extracted with a mobile phase. After dilution and filtration, the samples are analysed with ion chromatography and conductivity detection. This method has been validated for feedingstuffs. By spiking about 1500 mg/kg formate in pig feed and fish meal (n = 3), 95% recovery and 5% relative standard deviation was found. The limit of detection is 0.6 mg/kg feedingstuff. The limit of quantification is 2 mg/kg feedingstuff. The spiking in the validation of the method of analysis of feedingstuffs is performed at a lower level of formate - 1500 mg/kg feedingstuff – than the target range 6000-18000 mg/kg in feedingstuffs. This is not considered a serious problem, because spiking with a higher level presumably would improve the performance characteristics. The ion chromatographic method, which was submitted by the applicant for analysis of feedingstuff and of target tissues, is sufficiently validated and is considered suitable for routine control. It should be noted that the method for

analysis of Formi^{TM} LHS in feedingstuffs is not able to discriminate if the formic acid in the sample originates from Formi^{TM} LHS or other formic acid sources added to the feedingstuff (acidifiers or preservatives). Therefore, this method of analysis of formic acid in feedingstuff can not be used for verifying the maximum level of Formi^{TM} LHS in feedingstuff, when other sources of formic acid are present, and consequently, it is not considered appropriate for official control purposes. However, to our knowledge, no other routine analytical method would be able to discriminate the source of formic acid in feedingstuff.

While the method for analysis of Formi^{TM} LHS in premixtures has not been validated, this is not considered a serious problem, since the target inclusion levels for the additive in feedingstuff are quite high (in the percent range), and therefore comparable to the levels typically applied in premixtures.

The dossier contains information on the specification for contents of arsenic, cadmium, lead and mercury and the corresponding submitted methods of analysis (ICP-AES or AAS) have limits of quantification (0.02 mg/kg, 1 mg/kg, 2 mg/kg and 0.0003 mg/kg, respectively) well below the specifications . For other inorganics impurities in Formi^{TM} LHS, the methods of analysis (ICP-AES or AAS) are given with the corresponding limits of quantification. The anticaking agent silica, which is in the product at maximum 0.5%, can be analysed with ICP-AES (enclosure 2).

Further testing or validation is not considered necessary.

8. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

The applicant provided the CRL-FAA with the required reference samples.

The dossier provided by the applicant is divided into various documents structured according to the annex of Commission Directive 2001/79/EC apart from 2.1.5 Manufacturing process, which is missing.

Relevant information regarding section II (Identity, characterisation and conditions of use of the additive; methods of control) are given in the dossier, which is structured in the main part of the dossier (00_Section_II_Chem_Dossier_FormiTMLHS_May2004.doc) with enclosures and an expert report. Enclosures 27 and 47 were replaced by other information.

9. REFERENCES

(1) The EFSA Journal (2004) 139, 1-9. Opinion of the Scientific Panel on Additives and Products or Substances used in Animal Feed on a request from the Commission on the safety

and the efficiency of "Formi^{TM} LHS", based on potassium diformate, as a feed additive for sows in accordance with Council Directive 70/524/EEC. (Question no. EFSA-Q-2004-099).

(2) SCAN, 22 March 2001 http://europa.eu.int/comm./food/fs/sc/scan/out83bis_en.pdf

10. RAPPORTEUR LABORATORY

The Rapporteur Laboratory for this evaluation was

The Danish Plant Directorate, Lyngby, Denmark.