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

Hex-2(trans)-enal
(FEED-2021-1572; CRL/210060)



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

Dossier related to: **FEED-2021-1572 - CRL/210060**

Name of Product / Feed Additive: ***Hex-2(trans)-enal***

Active Agent (s): **Hex-2(trans)-enal**

Rapporteur Laboratory: **European Union Reference Laboratory for
Feed Additives (EURL-FA)
JRC Geel, Belgium**

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Date: **16/12/2022**

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Date: **16/12/2022**

EXECUTIVE SUMMARY

In the current application an authorisation is sought under Article 4 for *hex-2(trans)-enal* under the category/ functional group (1a) "technological additives"/"preservatives", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, the authorisation is sought for the use of the *feed additive* for all avian and all porcine species.

According to the Applicant, the *feed additive* has a minimum purity of 98 % (w/w) of *hex-2(trans)-enal*, which is the active substance of the product.

The *feed additive* is intended to be sprayed onto feed through *premixtures*. The Applicant proposed a maximum inclusion levels of *hex-2(trans)-enal* of 500 mg/kg *compound feed*.

For the quantification of *hex-2(trans)-enal* in the *feed additive* and *premixtures* the Applicant submitted two methods based on gas chromatography coupled with flame ionisation detection (GC-FID). These methods are based on generic gas chromatography assay recommended in Food Chemical Codex monograph for *hex-2(trans)-enal*.

For the quantification of *hex-2(trans)-enal* in *compound feed* the Applicant submitted a single-laboratory validated and further verified method based on gas chromatography with mass-spectrometry detection (GC-MS).

Based on the experimental evidence available the EURL recommends for official control the above mentioned GC-FID and GC-MS methods for the quantification of *hex-2(trans)-enal* in the *feed additive*, *premixtures* and *compound feed*, respectively.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

KEYWORDS

Hex-2(trans)-enal, technological additives, preservatives, all avian and all porcine species.

1. BACKGROUND

In the current application an authorisation is sought under Article 4(1) (new *feed additive*) for *hex-2(trans)-enal* under the category/ functional group (1a) "technological additives"/"preservatives", according to the classification system of Annex I of Regulation (EC) No 1831/2003 [1,2]. Specifically, the authorisation is sought for the use of the *feed additive* for all avian and all porcine species [1,2].

According to the Applicant, the *feed additive* is a pale yellow liquid with a minimum purity of 98 % (w/w) of *hex-2(trans)-enal*, which is the active substance of the product [3,4].

The *feed additive* is intended to be sprayed onto feed through *premixtures* [5]. The Applicant proposed a maximum inclusion levels of *hex-2(trans)-enal* of 500 mg/kg *compound feed* [5].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EU) 2015/1761, 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 *hex-2(trans)-enal* and their suitability to be used for official controls in the frame of the authorisation were evaluated.

3. EVALUATION

Description of the analytical methods for the determination of the active substance in the feed additive, premixtures, feedingstuffs and when appropriate water

For the quantification of the active substance (*hex-2(trans)-enal*) in the *feed additive* and *premixtures* the Applicant submitted two methods based on gas chromatography coupled with flame ionisation detection (GC-FID) [6,7]. These methods are based on generic gas chromatography assay recommended in Food Chemical Codex monograph for *hex-2(trans)-enal* [8].

Table 1. The performance characteristics of the GC-FID and GC-MS methods for the quantification of *hex-2(trans)-enal* in the *feed additive*, *premixtures* and *compound feed*, respectively.

	<i>Feed additive</i>	<i>Premixture</i>	<i>Compound feed</i>	
			<i>Validation</i>	<i>Verification</i>
Mass fraction, mg/kg	980000	169000	50 – 500	250 – 500
RSD_r, %	0.8	1.4	1.8 – 4.0	1.4 – 3.1
RSD_{ip}, %	1.0	1.5	14 ^(*)	2.5 – 6.3
R_{Rec}, %	95	101	82 – 100	84 – 92
Reference	[6,12]	[10,12]	[9,12]	[11,12]

RSD_r and RSD_{ip}: relative standard deviations for *repeatability* and *intermediate precision*, respectively; R_{Rec} - a *recovery rate*; (*) the RSD_{ip} value corresponds to *hex-2(trans)-enal* levels in feed at 50 mg/kg.

According to the specific protocols of the methods, the sample of the *feed additive* or *premixtures* is dissolved in dichloromethane or diethyl ether containing n-nonanal or methyl undecanoate as internal standards. Aliquots of the diluted solutions are analysed by GC. The quantification of the analyte is performed using calibration curves prepared from standard solutions of *hex-2(trans)-enal* containing the internal standards [6,7].

For the quantification of the active substance (*hex-2(trans)-enal*) in *compound feed* the Applicant submitted a single-laboratory validated and further verified method based on gas chromatography with mass-spectrometry detection (GC-MS) [9].

According to the method, the grinded sample is mixed with an internal standard (trans-2-heptenal) and further treated with dichloromethane. An aliquot of the extract is filtered through a membrane filter for further chromatographic analysis. The quantification of the analyte is performed using a calibration curve prepared from the standard solutions of *hex-2(trans)-enal* containing the internal standard [9].

The performance characteristics from the validation [6,9,10] and verification studies [11] (as partially re-calculated by the EURL [12]) of all above mentioned methods for the quantification of *hex-2(trans)-enal* in the *feed additive*, *premixtures* and *compound feed* are presented in Table 1.

In addition, the Applicant reported for the GC-MS method the limit of quantification (LOQ) of 10 mg *hex-2(trans)-enal* / kg *compound feed* [13].

Based on the experimental evidence available the EURL recommends for official control the above mentioned GC-FID and GC-MS methods for the quantification of *hex-2(trans)-enal* in the *feed additive*, *premixtures* and *compound feed*, respectively.

Methods of analysis for the determination of the residues of the additive in food (section 2.6.2 of the dossier - Annex II of Commission Regulation (EC) No 429/2008)

An evaluation of corresponding methods of analysis is not relevant for the present application.

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, as last amended by Regulation (EU) 2015/1761) is not considered necessary.

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the EURL recommends for official control: i) the two gas chromatography coupled with flame ionisation detection (GC-FID) methods (based on Food Chemical Codex monograph for *hex-2(trans)-enal*) for the quantification of *hex-2(trans)-enal* in the *feed additive* and *premixtures*; and ii) the single-laboratory validated and further verified method based on gas chromatography with mass-spectrometry detection (GC-MS) for the quantification of *hex-2(trans)-enal* in *compound feed*.

Recommended text for the register entry (analytical method)

For the quantification of *hex-2(trans)-enal* in the *feed additive* and *premixtures*:

- Gas chromatography with flame ionisation detection (GC-FID)

For the quantification of *hex-2(trans)-enal* in *compound feed*:

- Gas chromatography with mass-spectrometry detection (GC-MS)

5. DOCUMENTATION AND SAMPLES PROVIDED TO EURL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of *hex-2(trans)-enal* 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] *Forwarding of applications for authorisation of feed additives in accordance with Regulation (EC) No 1831/2003 – E-Submission Food Chain platform – <https://webgate.ec.europa.eu/esfc/#/applications/2346>
<https://open.efsa.europa.eu/questions/EFSA-Q-2022-00157>
- [2] *Application, Annex 1
- [3] *Technical dossier, Section II: 2.1.1. Name of the Additive
- [4] *Technical dossier, Section II: 2.1.5. Physical State of Each Form of the Additive
- [5] *Technical dossier, Section II: 2.5.1. Proposed mode of use in animal nutrition
- [6] *Technical dossier, Section II – Annex II-4

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- [7] *Supplementary information – Method_Anitox_T2H in Blend_TM-754
[8] Food Chemical Codex monograph "(E)-2-Hexen-1-al", FCC 7 (2010), p. 473
[9] *Technical dossier, Section II – Annex II-5
[10] *Supplementary information – Method Validation_T2H in Blends
[11] *Supplementary information – Method Validation_T2H in Feed
[12] *Supplementary information – EURL calculation performance characteristics
[13] *Supplementary information – FEED-2021-1572 (Hex-2(trans)-enal)
*Refers to Dossier no: FEED-2021-1572

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation is the European Union Reference Laboratory for Feed Additives, JRC, 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 (EU) 2015/1761.

8. ACKNOWLEDGEMENTS

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- Państwowy Instytut Weterynaryjny, Pulawy (PL)
- Thüringer Landesanstalt für Landwirtschaft (TLL). Abteilung Untersuchungswesen. Jena (DE)
- Centro di referenza nazionale per la sorveglianza ed il controllo degli alimenti per gli animali (CReAA), Torino (IT)
- Laboratori Agroalimentari, Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural. Generalitat de Catalunya, Cabrils (ES)