

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of vitamin B₆ as a feed additive for all animal species¹

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)^{2,3}

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ABSTRACT

Pyridoxine hydrochloride is a form of vitamin B₆, an essential micronutrient. It occurs in the body as pyridoxal 5'-phosphate. It serves as a coenzyme in transamination, decarboxylation and desamination reactions. It plays an important role in the metabolism of proteins (amino acids), fatty acids and carbohydrates, and in the synthesis of transmitters. Its major role is neuroprotection. Oral administration routes of pyridoxine hydrochloride via feed or water are considered as bioequivalent. Pyridoxine hydrochloride is considered to be safe for all animal species at the commercial use levels. The FEEDAP Panel concludes that the use of pyridoxine hydrochloride as a nutritional additive does not give rise to concern for consumers. The FEEDAP Panel, in the absence of data, cannot conclude on skin and eye irritation and skin sensitisation although the Panel notes that pyridoxine hydrochloride may cause photosensitisation. The data on physical properties of the specific formulation considered in this opinion would indicate that the risk of inhalation exposure is low. This conclusion could only apply to other formulations with similar physical properties. Pyridoxine occurs widely in nature (in most plant feed materials). Its use in animal nutrition is not expected to substantially increase the concentration in the environment. Therefore, a risk for the environment resulting from the use of pyridoxine in animal nutrition is not foreseen. Due to the long history of use and its established nutritional role in domestic animals, pyridoxine hydrochloride is regarded as an effective source of vitamin B₆.

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KEY WORDS

Nutritional additive, vitamins and pro-vitamins, vitamin B₆, pyridoxine hydrochloride

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SUMMARY

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of pyridoxine hydrochloride, supplied via feed or water, as a feed additive for all animal species.

Pyridoxine hydrochloride is a form of vitamin B₆, an essential micronutrient. It occurs in the body as pyridoxal 5'-phosphate. It serves as a coenzyme in transamination, decarboxylation and desamination reactions. It plays an important role in the metabolism of proteins (amino acids), fatty acids and carbohydrates, and in the synthesis of transmitters. Its major role is neuroprotection.

Oral administration routes of pyridoxine hydrochloride via feed or water are considered as bioequivalent.

Pyridoxine hydrochloride is considered to be safe for all animal species at the commercial use levels.

The FEEDAP Panel concludes that the use of pyridoxine hydrochloride as a nutritional additive does not give rise to concern for consumers.

The FEEDAP Panel, in the absence of data, cannot conclude on skin and eye irritation and skin sensitisation although the Panel notes that pyridoxine hydrochloride may cause photosensitisation. The data on physical properties of the specific formulation considered in this opinion would indicate that the risk of inhalation exposure is low. This conclusion could only apply to other formulations with similar physical properties.

Pyridoxine occurs widely in nature (in most plant feed materials). Its use in animal nutrition is not expected to substantially increase the concentration in the environment. Therefore, a risk for the environment resulting from the use of pyridoxine in animal nutrition is not foreseen.

Due to the long history of use and its established nutritional role in domestic animals, pyridoxine hydrochloride is regarded as an effective source of vitamin B₆.

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BACKGROUND

Regulation (EC) No 1831/2003⁴ establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7. Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Lohmann Animal Health GmbH & Co KG⁵ for authorisation of the product vitamin B₆ to be used as a feed additive for all animal species (category: nutritional additives; functional group: vitamins, pro-vitamins and chemically well-defined substances having similar effect) under the conditions mentioned in Table 1.

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 10(2) (re-evaluation of an authorised feed additive), and under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application.⁶ According to Article 8 of that Regulation, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. The particulars and documents in support of the application were considered valid by EFSA as of 22 February 2010.

Vitamin B₆ (pyridoxine hydrochloride) has been authorised without time limit under Council Directive 70/524/EEC⁷ for its use in all species as a nutritional additive.

The Scientific Committee on Food (SCF) expressed an opinion on the tolerable upper intake level of vitamin B₆ (SCF, 2000). The Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (CEF) issued an opinion on pyridoxal 5'-phosphate as a source for vitamin B₆ added for nutritional purposes in food supplements (EFSA, 2008).

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and the efficacy of the product vitamin B₆, when used under the conditions described in Table 1.

⁴ OJ L 268, 18.10.2003, p. 29.

⁵ Lohmann Animal health GmbH & Co. KG, Heinz Lohmann-Str. 4, 27472 Cuxhaven, Germany.

⁶ EFSA Dossier reference: FAD-2009-0045.

⁷ OJ C 50, 25.02.2004, p. 1.

Table 1: Description and conditions of use of the additive as proposed by the applicant

Additive	Vitamin B ₆
Registration number/EC No/No (if appropriate)	
Category(-ies) of additive	Nutritional Additive
Functional group(s) of additive	Vitamins, provitamins and chemically well defined substances having a similar effect

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Pyridoxine hydrochloride	C ₈ H ₁₁ NO ₃ HCl	Min 99 %	Ph. Euro. 6 th , monograph 0245

Trade name (if appropriate)	
Name of the holder of authorisation (if appropriate)	

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg/kg of complete feedingstuffs		
All animal species and categories	-	-	-	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	-
Specific conditions or restrictions for handling (if appropriate)	-
Post-market monitoring (if appropriate)	-
Specific conditions for use in complementary feedingstuffs (if appropriate)	-

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

1. Introduction

The term vitamin B₆ includes several inter-related substances: pyridoxine, pyridoxal, pyridoxamine and their respective 5'-phosphates. While pyridoxine is the predominant dietary form in plant products, pyridoxal and pyridoxamine are the principal forms present in animal tissues. The substance subject to the present application is pyridoxine hydrochloride, obtained by chemical synthesis.

Vitamin B₆ in the form of pyridoxine hydrochloride is a feed additive authorised for use in all species as a nutritional additive. The applicant asks for the re-evaluation of the use of pyridoxine hydrochloride as a nutritional additive for all animal species and categories and for a new use of this feed additive (use in water).

According to Commission Regulation (EC) No 1170/2009,⁸ vitamin B₆ in the form of pyridoxine hydrochloride, pyridoxine 5'-phosphate and pyridoxal 5'-phosphate can be used as a food supplement, and in the form of pyridoxine hydrochloride, pyridoxine 5'-phosphate and pyridoxine dipalmitate it can be added to food. Vitamin B₆ is also listed according to Commission Regulation (EU) No 37/2010 as a pharmacologically active substance in veterinary medicinal products and is not subject to maximum residue levels when used in food-producing animals.⁹

2. Characterisation

2.1. Characterisation of the additive

The additive that is the subject of this application is a white or almost white crystalline and practically odourless powder. Specification allows a minimum of 99 % purity. The analysis of five production batches showed a content of pyridoxine hydrochloride $\geq 99.5 \% \pm 0.1 \%$.¹⁰

Pyridoxine hydrochloride (synonyms: pyridoxol hydrochloride, vitamin B₆ hydrochloride, 2-methyl-3-hydroxy-4,5-bis(hydroxymethyl)pyridine hydrochloride, 5-hydroxy-6-methyl-3,4-pyridinedicarbinol hydrochloride, 3-hydroxy-4,5-dimethylol- α -picoline hydrochloride), with CAS number 58-56-0 and EINECS number 200-386-2, is the synthetic form of pyridoxine. The structural formula is presented in Figure 1.

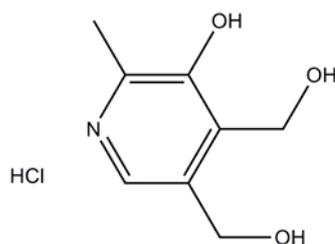


Figure 1. Structural formula of pyridoxine hydrochloride

The molecular formula of pyridoxine hydrochloride is C₈H₁₁NO₃•HCl, the molecular weight is 205.6 Da, with a melting point of 205 °C, shows a bulk density of 0.5–0.6 g/cm³ and is soluble in water (1 g dissolves in about 4.5 ml), sparingly soluble in acetone or ethanol, insoluble in ether and chloroform.

The applicant provided the results of the analysis of three batches. The known impurities 6-methyl-1,3-dihydrofuro[3,4-*c*]pyridine-7-ol and 5-(hydroxymethyl)-2,4-dimethylpyridin-3-ol (known as

⁸ OJ L 314, 1.12.2009, p. 36.

⁹ OJ L 15, 20.1.2010, p. 1.

¹⁰ Technical dossier/Section II and supplementary information June 2010.

impurity A and B, respectively) were below the limit of detection (2.5 mg/kg).¹¹ Residual organic solvents were conform with VICH thresholds for solvents of class 3, sulphated ashes were below 0.1 % (0.01-0.02 %) and heavy metals (expressed as lead) < 20 mg/kg. The pH of the batches was 2.67–2.70 and their melting points were 207.5–208.2 °C.¹²

Particle size examination showed that the mean particle diameter was 135 µm. No particles of 2.5 µm diameter or less were detected and the proportions of particles with diameters of less than 5, 10, 20 and 50 µm were 0.66 %, 1.83 %, 4.3 % and 15 % by volume, respectively.¹³ The results of a Stauber-Heubach test for dusting potential showed that the product did not produce any dust following shaking.¹⁴

2.2. Production process

The production process of pyridoxine hydrochloride is described in the technical dossier submitted by the applicant.

2.3. Stability and homogeneity

2.3.1. Shelf life of the additive

The applicant provided information on the recovery of pyridoxine hydrochloride (three batches) when kept in polyethylene bags at 25 °C (60 % relative humidity, RH) or at 40 °C (75 % RH) up to 48 or 6 months, respectively. The recovery values showed no reduction of the content of pyridoxine hydrochloride over time (48 months at 25 °C or 6 months at 40 °C).¹⁵

2.3.2. Stability of the additive when added to premixtures, feed and drinking water

The applicant provided information on the stability of pyridoxine hydrochloride (one batch) when incorporated in a premixture for chickens for fattening at 4000 mg/kg and kept in polyethylene bags at 20 °C (60 % RH) up to six months. The premixture contained vitamins and trace elements but did not contain choline chloride.¹⁶ Results showed no reduction on the content of pyridoxine hydrochloride.

Stability in premixtures containing choline chloride and trace elements showed a pyridoxine recovery of 92 % after one month of storage and 56 % recovery after six months (Whitehead, 2002). Recoveries of 93 % and 68 % after one and six months of storage, respectively, have been reported by Coelho (1996).

The retention of pyridoxine in poultry feedstuffs was inversely correlated with the temperature of feed processing (expansion), ranging from 96 % retention at a temperature of 93 °C x 30 s (or 110 °C x 5 s) to 80 % retention at 149 °C x 30 s (or 165 °C x 5 s) (Whitehead, 2002).

Stability information was provided on crystalline pyridoxine added to commercial fish feed (Marchetti et al., 1995). Initial concentrations of 80 mg/kg were reduced to 60 mg/kg after pelleting (by 25 %) and to 53 mg/kg after extrusion (by 34 %). Upon storage in paper bags at room temperature the concentrations of pyridoxine in pelleted fish feed were 61, 52 and 46 mg/kg after 30, 90 and 180 days, respectively, with a loss of 35 % after 90 days. In extruded feed, corresponding figures were 56, 48 and 38 mg/kg after 30, 90 and 180 days, respectively, with a loss of 40 % after 90 days.

The stability of the additive in water (one batch) was measured when added at 100 mg/l and kept for 24 hours at 15 °C.¹⁷ The results showed no modifications on the pyridoxine hydrochloride content. In

¹¹ Technical dossier/Section II/Annex 2.08.

¹² Technical dossier/Supplementary information June 2010/Annex 2.11.

¹³ Technical dossier/Supplementary information June 2010/Annex 2.13.

¹⁴ Technical dossier/Supplementary information October 2010/Annex 2.18.

¹⁵ Technical dossier/Supplementary information June 2010/Annex 2.12.

¹⁶ Technical dossier/Supplementary information June 2010/Annex 2.14.

¹⁷ Technical dossier/Section II.

a further study,¹⁸ three batches of the additive were tested for stability in water at 25 °C. Measurements after 6 and 24 hours were reported to have showed no change in the pyridoxine hydrochloride content.

2.3.3. Homogeneity

The applicant provided a statistical method (Jansen, 1992) to calculate the homogeneity of pyridoxine hydrochloride in feed (setting the maximum coefficient of variation (CV %) at 10 %). The calculations resulted in a CV % of 9.9 %.

Pyridoxine hydrochloride is highly soluble in water and therefore homogeneity in drinking water needs not to be demonstrated.

2.4. Conditions of use

Pyridoxine hydrochloride is to be used in all animal species and categories without a maximum limit. The active substance can be administered via feed or water. In the case of use in complete feed it is recommended to be incorporated through a premixture.

2.5. Evaluation of the analytical methods by the Community Reference Laboratory (CRL)

EFSA has verified the CRL report as it relates to the methods used for the control of vitamin B₆ in animal feed. The Executive Summary of the CRL report can be found in Appendix.

3. Safety

According to Regulation (EC) No 429/2008, tolerance, metabolism and residue, and toxicological studies are not required for vitamins, pro-vitamins and chemically defined substances having similar effects which are already authorised as feed additives under Directive 70/524/EEC⁶ and which do not have the potential to accumulate.

3.1. Safety for the target species

The National Research Council concluded in 1987 on pyridoxine tolerance that available evidence from dog and rat studies suggests that probably more than 1000 times the nutritional requirements (1–5 mg/kg feed) would have to be included in diets in order to produce signs of toxicity in those particular species (NRC, 1987). A more recent assessment characterises the high pyridoxine intake of dogs (50 mg/kg bw) as the LOAEL (EVM, 2002) instead of NOAEL (NRC, 1987). Recent studies on pigs (Böhmer and Roth-Maier, 2007) fed 20 times NRC requirements (NRC, 1998) and on laying hens (Leeson and Caston, 2003) fed five times NRC requirements (NRC, 1994) — originally designed to study tissue deposition — did not show any influence on performance parameters.

It is concluded that vitamin B₆ has a wide margin of safety for the target species (> 10-fold compared to the requirements).

3.2. Safety for the consumer

3.2.1. Absorption, distribution, metabolism and excretion of pyridoxine

The applicant made reference to the SCF document on tolerable intake levels for vitamins and minerals (SCF, 2000).

Although vitamin B₆ is generally considered as being rapidly eliminated, without significant potential for tissue retention above physiological levels, a few studies show deposition (in the +25 % to +50 % range) in edible tissues of farm animals fed supplemented feeds (Böhmer and Roth-Maier, 2007; Leeson and Caston, 2003).

¹⁸ Technical dossier/Supplementary information June 2010/Annex 2.16.

Overall, the limited database indicate that high supplemental levels lead to small increases in vitamin B₆ content in eggs, pig meat and pig liver as compared to the lowest supplemental levels.

3.2.2. Toxicological studies

The applicant referred to scientific opinions of SCF (2000) and EFSA (2008), with comprehensive overviews of the toxicological profile of vitamin B₆ including data and effects in humans and animals. The data includes a considerable number of long-term studies in laboratory animals and humans which have been reviewed by SCF (2000). Most of these studies considered the known neurological endpoints of vitamin B₆ toxicity, but showed some shortcomings in the design and protocol. Consequently, no NOAEL from animal studies could be derived.

Studies on laboratory rodents published after the SCF assessment indicate that dose levels higher than 100 mg/kg bw are required to elicit neurotoxicity (Arkaravichien et al., 2003; Perry et al., 2004; Hong et al., 2009). Some experimental studies indicate impairment of renal function and/or imbalances with other water-soluble vitamins (e.g. pantothenic acid) as factors enhancing vitamin B₆ neurotoxicity (Levine et al., 2002; Fukuwatari et al., 2009).

3.2.3. Assessment of consumer safety

3.2.3.1. Tolerable upper intake level (UL)

The SCF (2000) defined a UL of 25 mg/person per day in adults, based on the absence of neurological signs in humans at intakes of approximately 100 mg/person/day and an overall safety factor of 4, to account for long-term intake as well as deficiencies in the database. The SCF noted that there is no information pointing out unusually susceptible subgroups; the upper level intakes for children are based only on body weight differences compared to adults (SCF, 2000). No new data that would require the SCF opinion to be reviewed are available.

3.2.3.2. Consumer exposure

According to the SCF opinion (2000), the background intake of vitamin B₆ was in the range of 1.57 (UK) to 3.6 (Ireland) mg/person/day; most of the 97.5 percentile values were in the range of 3.01 (Netherlands) to 10.46 (UK) mg/person/day, showing no risk to exceed the UL from food sources. One exception was the value of 30.3 mg/day observed in women from Ireland, due to supplement use.

A more recent estimate of the intake of vitamin B₆ and of the most important food sources in ten EU countries was provided by Olsen et al. (2009), based on the European Prospective Investigation into Cancer and Nutrition. The overall intake was between 1.2 and 2.8 mg/person/day. Vegetables, fruits, dairy products, meat and potatoes were the most important dietary sources of vitamin B₆, with fish and eggs contributing in smaller amounts. For men, the main source was meat (17–34 % of intake) in most countries, whereas no single source could be identified as being the most important for women. Overall, foods of animal origin may make up to approximately 50 % of the total vitamin B₆ intake from food sources. The results of this study do not suggest that there is any risk of exceeding the UL for vitamin B₆ from dietary sources.

The information on vitamin B₆ metabolism and the limited data on retention in edible tissues and products (see Section 3.2.1), indicate that even supplemental levels in feeds far higher than the requirements (1–5 mg/kg feed) are highly unlikely to cause any concern about consumer's exposure exceeding the UL.

Therefore, the FEEDAP Panel concludes that the use of vitamin B₆ as nutritional additive in feeds is safe for consumers.

3.3. Safety for the user

3.3.1. Effects on the respiratory system

The additive that is the subject of this application (a powder of pyridoxine hydrochloride) was tested for dusting potential and analysed for particle size distribution. The particle size distribution was such that some (15 % fell below 50 µm) of the particles in any dust that might be formed would be of respirable size. However, the results of a Stauber-Heubach test showed that the product was non-dusting and therefore inhalation exposure is not expected.¹⁹ These results should not be extrapolated to all vitamin B₆ products as other chemical or physical forms of the vitamin might well form a respirable dust and may be potentially hazardous to users by inhalation.

3.3.2. Effects on the eyes and skin

No data are available on the skin sensitisation or irritancy to skin or eyes of the additive. In the absence of such data the additive is to be considered as a potential irritant to skin and eyes and potential skin sensitiser.

Results from an *in vitro* study (Sato et al., 1993) and a human case study (Morimoto et al., 1996) indicate that pyridoxine hydrochloride can be a skin photosensitiser.

3.4. Safety for the environment

Pyridoxine occurs widely in nature (in most plant feed materials). Its use in animal nutrition is not expected to substantially increase the concentration in the environment. Therefore, a risk for the environment resulting from the use of pyridoxine in animal nutrition is not foreseen.

4. Efficacy

According to Regulation (EC) No 429/2008, efficacy studies are not required for vitamins, pro-vitamins and chemically defined substances having similar effects which are already authorised as feed additives under Directive 70/524/EEC⁶ and which do not have the potential to accumulate.

Due to the long history of use and its established nutritional role in domestic animals, pyridoxine hydrochloride is regarded as an effective source of vitamin B₆.

Vitamin B₆ has been globally used in animal nutrition for decades. Data on requirement, allowances and recommendations for feed supplementation are easily accessible as standard literature for animal nutrition experts.

5. Post-market monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation²⁰ and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Oral administration routes of pyridoxine hydrochloride via feed or water are considered as bioequivalent.

Pyridoxine hydrochloride is considered to be safe for all animal species at the commercial use levels.

¹⁹ Technical dossier/Supplementary Information October 2010/Annex 2.18.

²⁰ OJ L 35, 8.2.2005, p. 1.

The FEEDAP Panel concludes that the use of pyridoxine hydrochloride as a nutritional additive does not give rise to concern for consumers.

The FEEDAP Panel, in the absence of data, cannot conclude on skin and eye irritation and skin sensitisation although the Panel notes that pyridoxine hydrochloride may cause photosensitisation. The data on physical properties of the specific formulation considered in this opinion would indicate that the risk of inhalation exposure is low. This conclusion could only apply to other formulations with similar physical properties.

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Due to the long history of use and its established nutritional role in domestic animals, pyridoxine hydrochloride is regarded as an effective source of vitamin B₆.

RECOMMENDATIONS

When giving a warrantee for premixture stability, the manufacturer should consider the effect of including choline chloride.

DOCUMENTATION PROVIDED TO EFSA

1. Vitamin B₆ as a feed additive for all animal species. October 2009. Submitted by Lohmann Animal Health GmbH & Co KG.
2. Vitamin B₆ as a feed additive for all animal species. Supplementary information. June 2010 and October 2010. Submitted by Lohmann Animal Health GmbH & Co KG.
3. Evaluation report of the Community Reference Laboratory for Feed Additives on the methods(s) of analysis for vitamin B₆.
4. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the Community Reference Laboratory for Feed Additives on the Method(s) of Analysis for vitamin B₆

In the current application authorisation is sought for *Vitamin B6* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *pyridoxine hydrochloride (Vitamin B6)* for all animal species and categories. The feed additive is an almost white crystalline powder with a minimum content of 99% of pyridoxine hydrochloride. Vitamin B6 is intended to be incorporated in feedingstuffs via *premixture*. Due to excellent solubility in water the additive can be used directly for water applications. The applicant does not propose any maximum or minimum concentration of feed additive in feed or water. Furthermore no limits are set in previous regulations.

For the determination of the active substance *pyridoxine hydrochloride (Vitamin B6)* in the *feed additive* the applicant proposes the titration method described in the European Pharmacopoeia (Ph.Eur.6th, monograph 0245). The CRL-FA considers this method suitable to be used within the frame of official control.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures* the applicant proposes a method of the Association of German Agricultural Analytical and Research Institutes (VDLUFA, Germany). The method is based on High Performance Liquid Chromatography (HPLC) coupled to an UV detector and was ring-trial validated on premixtures containing the target analyte at concentration from 627 to 11530 mg/kg. The following performance characteristics were reported:

- relative standard deviation for *repeatability* (RSD_r) ranging from 2.6 to 3.4% , and
- relative standard deviation for *reproducibility* (RSD_R) ranging from 4 to 5.1%.

Based on these acceptable performance characteristics the CRL considers this method suitable for the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures* within the concentration range covered by the collaborative study. Therefore the CRL-FA recommends this VDLUFA Bd.III, 13.9.1 method for official control to determine *pyridoxine hydrochloride (Vitamin B6)* in *premixtures*.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *feedingstuffs* and in *water*, the applicant did not submit any method. Therefore the CRL-FA cannot evaluate nor recommend any method for the determination of the active substance in these matrices.

No further testing or validation is required.