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## Safety and efficacy of betaine anhydrous for food-producing animal species based on a dossier submitted by AB Vista

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### Abstract

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 betaine anhydrous produced [REDACTED] as a nutritional additive to be used in feed for all food-producing animal species except rabbits. Glycine betaine (betaine) acts as a methyl group donor in transmethylation reactions in organisms. Betaine occurs in numerous tissues of vertebrates as an osmolyte ensuring osmoprotection. Betaine anhydrous (97%, 96% or 91%) is considered safe for the target species at a maximum supplementation rate of 2,000 mg betaine/kg complete feed. The use of betaine anhydrous (97%, 96% and 91%) as a feed additive up to a supplementation of 2,000 mg/kg complete feed does not pose concerns to consumer safety. The betaine liquid product contains a high proportion of unknown material (about 30% on dry matter basis). The FEEDAP Panel therefore cannot conclude on the safety of the liquid form of the additive for the target species and the consumer. In the absence of data, the FEEDAP Panel cannot conclude on the safety for the user. The supplementation of feed with betaine anhydrous does not pose a risk to the environment. Betaine has a potential to become efficacious in food-producing animal species and categories when administered via feed, especially when methyl groups from methionine or choline are limiting. The FEEDAP Panel made a recommendation on the use of the additive in premixtures without choline chloride.

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## 1. Introduction

### 1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003<sup>1</sup> 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.

The European Commission received a request from AB Vista<sup>2</sup> for authorisation of the product betaine anhydrous, when used as a feed additive for all animal species (category: nutritional additive; functional group: vitamins, provitamins and chemically well-defined substances having similar effect).

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 4(1) (authorisation of a feed additive or new use of a feed additive). The particulars and documents in support of the application were considered valid by EFSA as of 22 May 2015.

According to Article 8 of Regulation (EC) No 1831/2003, 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. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product betaine anhydrous, when used under the proposed conditions of use (see Section 3.1.6).

### 1.2. Additional information

Betaine anhydrous and betaine hydrochloride [redacted] and betaine anhydrous produced from [redacted] are authorised as feed additives for all animal species (Commission Regulation (EU) 2015/1060).<sup>4</sup> No maximum contents of betaine in feeds are established in the European Union (EU).

The compound under assessment is betaine anhydrous produced [redacted].

The Panel on Dietetic Products, Nutrition and Allergies (NDA) issued two opinions related to the use of betaine as a novel food in the EU (EFSA, 2005; EFSA NDA Panel, 2017). The NDA Panel expressed one opinion on the substantiation of health claims related to betaine and contribution to normal homocysteine metabolism (ID 4325) pursuant to Article 13(1) of Regulation (EC) No 1924/2006<sup>5</sup> (EFSA NDA Panel, 2011b). The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) issued three opinions on the safety and efficacy of betaine for all animal species (EFSA FEEDAP Panel, 2013a,b,c).

Betaine is used in veterinary medicine (oral or injectable) as a hepatoprotectant in cattle, pigs, goats, sheep and horses (EMA, 1997). Betaine anhydrous is also designated as an orphan medicinal product (EU/3/01/45) and is authorised (EMA, 2007) for the adjunctive treatment of hyperhomocysteinaemia/homocystinuria in humans (oral administration; see also Sharma et al., 2015).<sup>6</sup> Betaine is not described in the European Pharmacopoeia.

## 2. Data and methodologies

### 2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier<sup>7</sup> in support of the authorisation request for the use of betaine anhydrous as a feed additive.

<sup>1</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.

<sup>2</sup> AB Vista, Unit 3, Marlborough Businee Park, Blenheim Road, SN8 4AN, Marlborough, United Kingdom.

<sup>3</sup> The holder of the authorisation is Trouw nutrition International BV.

<sup>4</sup> Commission implementing Regulation (EU) 2015/1060 of 2 July 2015 concerning the authorisation of betaine anhydrous and betaine hydrochloride as feed additives for all animal species. OJ L 174, 3.7.2015, p. 3.

<sup>5</sup> Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. OJ L 404, 30.12.2006, p. 9.

<sup>6</sup> EMA Product No. EMEA/H/C/000678; EU No. EU/1/06/379/001

<sup>7</sup> FEED dossier reference: FAD-2014-0031.

The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008<sup>8</sup> and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the betaine in animal feed. The Executive Summary of the EURL report can be found in Annex A.<sup>9</sup>

## 2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of betaine anhydrous is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012a), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011a), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012b) and Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012c).

## 3. Assessment

The applicant is seeking authorisation of the use of betaine anhydrous produced [REDACTED] as an additive to feed for food-producing animal species (excluding rabbit) and categories. The substance is intended as a nutritional additive under the functional group vitamins, pro-vitamins and chemically well-defined substances having similar effects.

### 3.1. Characterisation

#### 3.1.1. Manufacturing process

Betaine occurs naturally in sugar beets, cereals (e.g. wheat) and some other feedstuffs. The betaine under application is produced [REDACTED]

Betaine is produced [REDACTED]

#### 3.1.2. Characterisation of the active substance

Betaine [REDACTED]

<sup>8</sup> Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

<sup>9</sup> The full report is available on the EURL website: [https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0031-betaine\\_anhydrous.pdf](https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0031-betaine_anhydrous.pdf)



**Figure 1:** [Redacted]

### 3.1.3. Characterisation of the additives

The additive betaine anhydrous is supplied in four different forms, three crystalline forms of betaine anhydrous (containing 97%, 96% and 91% betaine) and one liquid formulation (betaine liquid, 32%).<sup>11</sup>

#### 3.1.3.1. Characterisation of betaine anhydrous

Betaine anhydrous 97% is a [Redacted] with faint molasses odour, with a pH of 7–10 in 5% water solution,<sup>12</sup> [Redacted]. It is fully soluble in water, soluble in methanol and ethanol.

Betaine anhydrous contains by specification  $\geq 97\%$  betaine,  $\leq 1.5\%$  moisture and  $\leq 1\%$  ash.<sup>13</sup> Analysis of five batches showed a mean betaine content of 98.1% (range 97.2–98.7%). Moisture was on average 0.61% (range 0.54–0.70%). No data on ash content were given.<sup>14</sup> The amount of identified matter was on average 98.7% on dry matter basis.

The levels of heavy metals and arsenic were measured in five batches and were  $< 0.01$  mg/kg for mercury and cadmium,  $< 0.1$  mg/kg for arsenic and for lead ranged from  $< 0.02$  to 0.37 mg/kg. Dioxin-like polychlorinated biphenyls (PCBs, five batches) ranged from 0.03 to 0.3 ng WHO-PCB-TEQ (World Health Organization-PCB-toxic equivalent)/kg and polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/F, four batches) ranged from 0.07 to 0.33 ng WHO-PCDD/F-TEQ/kg. The sum of PCDD/F and PCB (four batches) ranged from 0.11 to 0.46 ng WHO-PCDD/F-PCB-TEQ/kg. Pesticides (multiresidue) were also analysed and were found below the limits of quantification (LOQ, 0.01 mg/kg). The absence of microbiological contamination was demonstrated in two batches of the additive (*Escherichia coli*  $< 10$  colony forming units (CFU)/g and *Salmonella* spp. not detected in 25 g samples).<sup>15</sup>

The dusting potential of one batch of betaine anhydrous 97% (DIN 55992 method) was 0.06 g/m<sup>3</sup>.<sup>16</sup> The particle size distribution (laser diffraction) shows that 10% (v/v) of particles have a diameter of  $< 149$   $\mu\text{m}$ .<sup>17</sup> The fractions of particles of  $< 100$ ,  $< 50$  and  $< 10$   $\mu\text{m}$  diameter were not specified. The average particle size was 390  $\mu\text{m}$  diameter and 80% of the particles have a size between 149 and 650  $\mu\text{m}$  diameter.

#### 3.1.3.2. Characterisation of the formulated crystalline products

Betaine anhydrous 96% and 91% are derived from betaine anhydrous 97% [Redacted], respectively. Three batches of [Redacted] were analysed for dioxins and dioxin-like PCBs and the results ranged from 0.149 to 0.660 ng WHO-PCDD/F-PCB-TEQ/kg.<sup>18</sup> One of these batches was further analysed for heavy metals (cadmium (0.053 mg/kg), lead (0.661 mg/kg) and mercury (below limit of quantification (LOQ))<sup>19</sup>) and arsenic (0.086 mg/kg); microbial contamination by *E. coli* ( $< 10$  CFU/g) or *Salmonella* spp. (absent in 25 g) and pesticides (all pesticides analysed  $< \text{LOQ}$ ).<sup>20</sup>

<sup>11</sup> Technical dossier/Section II/Annex II.8.

<sup>13</sup> Technical dossier/Section II/Annex II 8.

<sup>14</sup> Technical dossier/Section II/Annex II 6b.

<sup>15</sup> Technical dossier/Section II/Annex II 6a.

<sup>16</sup> Technical dossier/Supplementary information July 2016/Annex 2.

<sup>17</sup> Technical dossier/Section II/Annex II 13.

<sup>18</sup> Technical dossier/Section II/Annex II.6a.

<sup>19</sup> LOQ of mercury was 0.01 mg/kg.

<sup>20</sup> LOQ of pesticides was 0.01 mg/kg.

### Betaine anhydrous 96%

Betaine anhydrous 96% is a [REDACTED], with faint molasses odour, with a pH of 7–10 in 5% water solution.<sup>21</sup>

Betaine anhydrous 96% contains by specification  $\geq 96\%$  betaine anhydrous, [REDACTED],  $\leq 1.5\%$  moisture and  $\leq 1.5\%$  ash.<sup>13</sup> Analysis of five batches showed a mean betaine content of 97.1% (range 96.6–97.8%). Moisture was on average 1.02% (range 0.95–1.08%).<sup>14</sup> No data were available on [REDACTED] or ash content. The amount of identified material was on average 98.1% on dry matter basis (without calcium stearate).

The dusting potential of one batch of betaine anhydrous 96% was 0.37 g/m<sup>3</sup> (DIN 55992).<sup>16</sup> The particle size distribution (laser diffraction) shows that 10% (v/v) of the particles have a diameter < 141  $\mu\text{m}$ .<sup>17</sup> The fractions of particles of < 100, < 50 and < 10  $\mu\text{m}$  diameter were not specified. The average particle size is 410  $\mu\text{m}$  of diameter and 80% of the particles have a diameter of 141–709  $\mu\text{m}$ .

### Betaine anhydrous 91%

Betaine anhydrous 91% is a [REDACTED], with faint molasses odour, with a pH of 7–10 in 5% water solution.<sup>22</sup>

Betaine anhydrous 91% contains by specification  $\geq 91\%$  betaine anhydrous, [REDACTED],  $\leq 1.5\%$  moisture and  $\leq 2\%$  ash.<sup>13</sup> Analysis of five batches showed a mean betaine content of 92.6% (range 92.3–93.2%). Moisture was on average 0.88% (range 0.82–1.01%).<sup>14</sup> No data were available on [REDACTED] or ash content. The amount of identified material was on average 93.5% on dry matter basis.

The dusting potential (one batch analysed by the DIN 55992 method) was 1.04 g/m<sup>3</sup>.<sup>23</sup> The particle size distribution (laser diffraction) shows that 25% (v/v) of the particles have a diameter < 189  $\mu\text{m}$  and 10% of the particles a diameter < 6.2  $\mu\text{m}$ .<sup>17</sup> The average particle size is 347  $\mu\text{m}$  diameter and 80% of the particles have a diameter of 6–641  $\mu\text{m}$ .

#### 3.1.3.3. Characterisation of betaine liquid 32%

Betaine liquid 32% is a [REDACTED] with a pH of 7.5–9.5 and a [REDACTED].<sup>24</sup>

Betaine liquid contains by specification  $\geq 32\%$  betaine anhydrous,  $\leq 45\%$  water and  $\leq 10\%$  ash.<sup>25</sup> Analysis of six batches showed a mean betaine content of 38.8% (range 35.3–42.0%). Water (two batches analysed) ranged from 42% to 47% and ash content (analysed in one single batch) was 0.54%.<sup>26</sup> The amount of identified material in one batch analysed was about 83.3% on 'as is' basis (68.5% on a dry matter basis).<sup>27</sup>

Impurities were measured in three batches.<sup>28</sup> Regarding heavy metals, mercury was < 0.01 mg/kg and cadmium and lead were < 0.02 mg/kg. Arsenic was < 0.05 mg/kg. One batch was tested for mycotoxins: aflatoxins (B1, B2, G1, G2 and total aflatoxins) were < 0.01  $\mu\text{g}/\text{kg}$ , ochratoxin was < 0.5  $\mu\text{g}/\text{kg}$ , zearalenone was 32  $\mu\text{g}/\text{kg}$ , deoxynivalenol was < 50  $\mu\text{g}/\text{kg}$ , toxins T2 and HT2 were < 3  $\mu\text{g}/\text{kg}$  and fumonisins B1 and B2 were < 10  $\mu\text{g}/\text{kg}$ . One batch was tested for dioxin-like PCBs, dioxins and furans (0.178 ng WHO-PCCD/F-PCB-TEQ/kg) and another only for dioxin-like PCBs (0.11 ng WHO- PCB-TEQ/kg). Melamine was < 0.05 mg/kg in two batches. Pesticides (multiresidue) were also analysed and were found to be below the LOQs. As refers microbial contamination of the product, *Salmonella* spp. was absent (25 g samples) and *E. coli*, coliforms, aerobic and anaerobic counts were < 10 CFU/g (three batches).

For the purpose of the assessment, the Panel considers that betaine anhydrous 96% and 91% can be regarded as similar to the active substance 97% (only [REDACTED] is added), whereas the betaine liquid 32% is seen as a different product as it is not sufficiently characterised (about 10% unidentified material).

<sup>23</sup> Technical dossier/Section II/Annex II 14 and supplementary information July 2016/Annex 2.

<sup>25</sup> Technical dossier/Section II/Annex II 8 and supplementary information July 2016/Annex 7.

<sup>26</sup> Technical dossier/Section II/Annexes II 7a, 7b and supplementary information July 2016/Annex 6.

<sup>27</sup> Technical dossier/Supplementary information July 2016/Annex 6.

<sup>28</sup> Technical dossier/Section II/Annex II 7a and Supplementary information July 2016/Annexes 8a and 8b.

### 3.1.4. Stability and homogeneity

#### 3.1.4.1. Shelf life

The shelf life of betaine anhydrous 97% (three batches),<sup>29</sup> betaine anhydrous 96% (two batches) and betaine anhydrous 91% (two batches) was studied when stored in plastic bags at 20°C for 36 (betaine anhydrous 97%) or 24 months (betaine anhydrous 96% and 91%). Losses were only observed in one batch of betaine anhydrous 91% and amounted to 0.9%.

The shelf life of one batch of the liquid form was tested when stored in plastic bottles at 20°C for 2 years and a half. No loss was observed.<sup>30</sup>

#### 3.1.4.2. Stability in premixtures

No data were provided on the stability in premixtures of the betaine products under assessment.

The applicant submitted a report (Kampf et al., 2012).<sup>31</sup> It contains data on the stability of one batch of betaine anhydrous 93% (origin not described) in a vitamin/mineral premixture for piglets (without choline chloride). It was stored at 20 and at 37°C for 3 months (packaging not described). The losses were 21% and 18%, respectively.

#### 3.1.4.3. Stability in feedingstuffs

The stability of one batch of betaine anhydrous 97% was tested in a complete feed for chickens for fattening (mash and pellet).<sup>32</sup> The basal diet consisted of maize, soybean meal and rice. The product was supplemented at 1.2 g betaine/kg and stored at 23°C for 3 months. The background content of betaine and the packaging used during storage were not specified. Total betaine was analysed. Losses of up to 7% were detected in mash and up to 6% in pelleted feed at the end of the storage period. The effect of pelleting was not reported.

The applicant submitted some data on the stability of the liquid form in feedingstuffs.<sup>33</sup> These data, however, could not be considered: as repository samples were tested, it was not possible to compare the betaine concentration at the beginning and at the end of the storage period for the same batch.

#### 3.1.4.4. Homogeneity

The capacity of one batch of betaine anhydrous 97% to homogeneously distribute in feed was tested in a mash feed for chickens for fattening supplemented at 1 g/kg feed. The coefficient of variation of 10 subsamples was 10.1%.<sup>34</sup>

### 3.1.5. Physicochemical incompatibilities in feed

No physicochemical incompatibilities or interactions have been reported between betaine anhydrous and feed materials, feed additives or carriers when the additive was added to premixtures and feed. No such incompatibilities or interactions are expected.

### 3.1.6. Conditions of use

Betaine (all four forms) is intended for use in feed for all food-producing animal species (except rabbits) and categories. The crystal forms can be administered directly to feedstuffs or via premixtures. The liquid form is to be used directly in feedingstuffs.<sup>35</sup>

For solid betaine anhydrous, the applicant reports inclusion levels (minimum to maximum recommended doses) of 100–2,000 mg/kg in poultry and pigs, 1,000–15,000 mg/kg complete feed for fish, 5,000–50,000 mg/head and day for cattle (corresponding to 560–5,580 mg/kg feed in cattle for fattening and to 223–2,230 mg/kg feed for cows), 4,000–6,000 mg/head and day for sheep (corresponding to 2,980–4,460 mg/kg feed), 2,000–10,000 mg/head and day for goats (corresponding to 1,490–7,440 mg/kg feed) and 10,000–20,000 mg/head and day for horses (corresponding to 1,120–2,232 mg/kg feed).

<sup>29</sup> Technical dossier/Section II/Annex II.19 and Supplementary information July 2016/Annex 3.

<sup>30</sup> Technical dossier/Section II/Annex II.19.

<sup>31</sup> Technical dossier/Supplementary information January 2016/Annex 2.

<sup>32</sup> Technical dossier/Supplementary information January 2016/Annexes 3 and 4; supplementary information July 2016/Annex 4.

<sup>33</sup> Technical dossier/Supplementary information July 2016/Annex 9.

<sup>34</sup> Technical dossier/Annex II.20.

<sup>35</sup> Technical dossier/Section 2.5.1.



For the betaine anhydrous liquid form, the applicant reports minimum and maximum recommended levels three times higher than for the solid forms.

## 3.2. Safety

### 3.2.1. Safety for the target species

The applicant submitted a tolerance study in weaned piglets performed with a betaine anhydrous  $\geq 97\%$  obtained from chemical synthesis.<sup>36</sup> This study has already been assessed by the FEEDAP Panel in the context of the re-evaluation of betaine as nutritional additive for all animal species (EFSA FEEDAP Panel, 2013a,b,c).

Based on this study, the Panel concluded that 'Betaine is safe for piglets at the maximum supplementation rate of 2,000 mg/kg complete feed with a margin of safety below 5'. This conclusion was extended to all pigs and extrapolated to all animal species and categories.

The FEEDAP Panel considers that the conclusions of the previous opinion apply to the crystalline forms of betaine anhydrous (containing 97%, 96% and 91% betaine). Considering the uncertainty in the characterisation of the liquid product (betaine liquid 32%), the FEEDAP Panel cannot conclude on the safety of the liquid form of the additive for the target animals.

### 3.2.2. Safety for the consumer

No toxicological studies were provided. The crystalline forms of betaine anhydrous (91%, 96% and 97%) are well characterised. The liquid form, however, has about 10% of its composition not characterised and genotoxicity studies were not provided. Considering the uncertainty in the characterisation of the liquid product (betaine liquid 32%), the FEEDAP Panel cannot conclude on its safety for the consumer.

In its previous opinion, the FEEDAP Panel considered unlikely that the use of betaine anhydrous as a feed additive up to a supplementation of 2,000 mg/kg complete feed would substantially increase consumer exposure. The FEEDAP Panel concluded that the use of betaine as feed additive up to a supplementation of 2,000 mg/kg complete feed is unlikely to pose any concern for consumer safety (EFSA FEEDAP Panel, 2013a,b,c).

The conclusions were based on the following considerations: (i) betaine is rapidly absorbed in the intestine and metabolised in the liver; (ii) in the tolerance study with piglets described above, no bioaccumulation of betaine in the liver was observed at the highest proposed use level (2,000 mg/kg feed), however, a twofold increase was observed at the intermediate and highest dose (5X and 10X); (iii) betaine is naturally present in foodstuffs; (iv) dietary choline is irreversibly oxidised to betaine in the body and contributes to the endogenous betaine pool; (v) no adverse effects were reported in human intervention studies (EFSA, 2005).

The recent assessment on the safety of betaine as novel food reviewed new toxicological evidence (EFSA NDA Panel et al., 2017). A chronic rat study indicated the BMDL<sub>05</sub> for increased platelet count of 131 mg/kg body weight (bw) as a reference point for toxicological effects. Human studies carried out with betaine supplementation indicated an increase in total and low-density lipoprotein (LDL)-cholesterol concentrations: a supplemental intake of 6 mg/kg bw per day for adults and up to 9.2 mg/kg bw per day for adolescents (10–14 years), in addition to the background exposure. The background dietary intake (up to about 830 mg/day; i.e. 12 mg/kg bw per day for adults and up to 19 mg/kg bw per day for adolescents aged 10–14 years) is not known to be associated with adverse effects (EFSA NDA Panel et al., 2017). The FEEDAP Panel considers that these additional findings do not change the conclusions on the safety for the consumer of the use of betaine anhydrous in animal nutrition.

Therefore, the FEEDAP Panel considers that the conclusions of the previous opinions apply to the crystalline forms of betaine anhydrous (containing 97%, 96% and 91% betaine) when used as feed additives at the maximum supplementation rate of 2,000 mg/kg complete feed for all food-producing animals except rabbits.

### 3.2.3. Safety for the user

No data have been provided on the effects of the additive on eyes and skin or on the respiratory system or on the sensitising potential. The solid forms of the additive have the potential to generate

<sup>36</sup> Technical dossier/Supplementary information January 2016/Annexes 5, 6 and 7.

dust and therefore exposure by inhalation cannot be excluded. In the absence of data, the FEEDAP Panel cannot conclude on the safety for the user.

### 3.2.4. Safety for the environment

Betaine is naturally present in the environment and its use in animal nutrition is not expected to substantially increase its concentration in the environment. Consequently, the supplementation of feed with betaine anhydrous does not pose a risk to the environment.

### 3.3. Efficacy

In its previous opinions on betaine anhydrous as a feed additive for all animal species, the FEEDAP Panel concluded that: (i) due to its established nutritional role in domestic animals, betaine anhydrous is regarded as an effective source of betaine and (ii) betaine has the potential to become efficacious in all animal species and categories when administered via feed (EFSA FEEDAP Panel, 2013a,b,c), especially when methyl groups from methionine or choline are limiting.

The FEEDAP Panel considers that the conclusions reached in the previous assessments apply to the current application.

### 3.4. 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 Regulation<sup>37</sup> and Good Manufacturing Practice.

## 4. Conclusions

Betaine anhydrous (97%, 96% or 91%) is considered safe for the target species at a maximum supplementation rate of 2,000 mg betaine/kg complete feed.

The use of betaine anhydrous (97%, 96% and 91%) as a feed additive up to a supplementation of 2,000 mg/kg complete feed does not pose concerns to consumer safety.

The betaine liquid product contains a high proportion of unknown material (about 30% on dry matter basis). The FEEDAP Panel therefore cannot conclude on the safety of the liquid form of the additive for the target species and the consumer.

In the absence of data, the FEEDAP Panel cannot conclude on the safety for the user.

The supplementation of feed with betaine anhydrous does not pose a risk to the environment.

Betaine has a potential to become efficacious in food-producing animal species and categories when administered via feed, especially when methyl groups from methionine or choline are limiting.

## 5. Recommendation

Since the stability of betaine in a vitamin–trace element premixture containing choline chloride has not been demonstrated, its use in premixtures containing choline chloride should be avoided.

### Documentation provided to EFSA

- 1) Betaine anhydrous [REDACTED]. September 2014. Submitted by AB Vista.
- 2) Betaine anhydrous [REDACTED]. Supplementary information. January 2016. Submitted by AB Vista.
- 3) Betaine anhydrous [REDACTED]. Supplementary information. July 2016. Submitted by AB Vista.
- 4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Betaine anhydrous.
- 5) Comments from Member States.

<sup>37</sup> Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

## Chronology of betaine anhydrous of AB vista

Date	Event
16/09/2014	Dossier received by EFSA
16/09/2014	Reception mandate from the European Commission
22/05/2015	Application validated by EFSA – Start of the scientific assessment
02/07/2015	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation, stability and safety for the target species</i>
21/08/2015	Reception of the Evaluation report of the European Union Reference Laboratory for Feed Additives
22/08/2015	Comments received from Member States
21/01/2016	Reception of supplementary information from the applicant - Scientific assessment re-started
10/03/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation of the additives and stability.</i>
11/07/2016	Reception of supplementary information from the applicant - Scientific assessment re-started
19/08/2016	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. <i>Issues: characterisation of the additives, stability and safety for the user.</i>
19/01/2018	No supplementary information had been received from the applicant and the scientific opinion was finalised with the data available in the dossier - Scientific assessment re-started
12/06/2018	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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## Abbreviations

CAS	Chemical Abstracts Service
CFU	colony forming unit
DIN	German Institute for Standardization
EINECS	European Inventory of Existing Chemical Substances
EMA	European Medicines Agency
EURL	European Union Reference Laboratory
FEEDAP	EFSA Panel on Additives and Products or Substances used in Animal Feed
HPLC-RI	high-performance liquid chromatography with refractive index detector
ICUMSA	International Commission for Uniform Methods of Sugar Analysis
IUPAC	International Union of Pure and Applied Chemistry
LDL	low-density lipoprotein
LOD	limit of detection
LOQ	limit of quantification
NDA	EFSA Panel on Dietetic Products, Nutrition and Allergies
NOAEL	no observed adverse effect level
PCB	polychlorinated biphenyl
PCDD/F	polychlorinated dibenzo- <i>p</i> -dioxins, polychlorinated dibenzofurans
pH	hydrogen potential
pKa	logarithm of the reciprocal of an acid dissociation constant
TEQ	toxic equivalents
UL	upper intake level
WHO	World Health Organization

## Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Methods of Analysis for Betaine Anhydrous

In the current application authorisation is sought for Betaine anhydrous, under article 4, for 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. Authorisation is sought for all animal species and categories. The feed additive is produced from [REDACTED]. According to the Applicant, Betaine anhydrous is to be marketed in two forms: as a brown liquid with a minimum content of 32% Betaine anhydrous; or a free flowing powder with a minimum purity of 91%. The liquid form is intended to be sprayed directly on feedingstuffs only, while the solid form is to be added into feedingstuffs directly or through premixtures. The Applicant proposed minimum contents of Betaine anhydrous in feedingstuffs of 0.1 g/kg for poultry and pigs and 1 g/kg for fish, while for other animal species and categories minimum daily doses "per head" were proposed ranging from 2 to 10 g per head/day. No maximum concentrations in feedingstuffs were specified.

For the quantification of Betaine anhydrous in the feed additive, premixtures and feedingstuffs the Applicant submitted an official ICUMSA's method (International Commission for Uniform Methods of Sugar Analysis) based on High Performance Liquid Chromatography with Refractive Index detector (HPLC-RI). This method is intended for the quantification of betaine in beet molasses. However, no experimental data were provided by the Applicant to demonstrate the applicability of the ICUMSA method for the quantification of Betaine anhydrous in feed additive, premixtures and feedingstuffs.

Nevertheless, the EURL previously evaluated several analytical methods in the frame of the "Betaine anhydrous and related compounds" dossiers, including a Betaine anhydrous produced from [REDACTED]. This feed additive is presently authorised by Commission Implementing Regulation (EU) 2015/1060 of 2 July 2015. The conclusions of the EURL included therein are considered valid for the purpose of the current application.

"For the quantification of betaine in feed additive, premixtures and feedingstuffs, Applicant submitted a single-laboratory validated and further verified method based on HPLC-RI. The following performance characteristics were reported:

- a precision (repeatability and intermediate precision) ranging from 0.1 to 0.8% for the feed additive, or from 4.3 and 8.6% for premixtures and feedingstuffs;
- a recovery rate (RRec) ranging from 94 to 107% for premixtures and feedingstuffs; and a limit of quantification (LOQ) of 70 mg/kg feedingstuffs."

(extract from the EURL report on Betaine, 2011)

Based on the performance characteristics available, the EURL recommends for official control the single-laboratory validated and further verified method, using HPLC-RI, to quantify Betaine anhydrous (expressed as total betaine) in the feed additives, premixtures, feedingstuffs within the concentration range covered by the experimental data.

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.