

SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of propionic acid, sodium propionate, calcium propionate and ammonium propionate for all animal species¹

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

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ABSTRACT

The maximum safe level of propionic acid for poultry is 10 g/kg complete feed, for pigs 30 g/kg complete feed. The corresponding safe concentrations in water for drinking would be 4 and 10 g/L, respectively. Ruminants show a high tolerance to propionic acid. Differences in the safety of propionates and propionic acid are not expected. Propionic acid, sodium propionate and calcium propionate are authorised in the EU for use in food. Ammonium propionate, not authorised as food additive, will essentially share the metabolic pathways of the other propionates. Propionic acid occurs endogenously as a by-product of normal intermediate metabolism and consequently residues in meat, milk or eggs are expected to be negligible. The use of propionic acid and its salts in animal nutrition is therefore considered of no concern for the safety of consumers. Propionic acid and sodium propionate are corrosive to the skin, the eye and mucous membranes; calcium propionate is not classified as an irritant. Propionic acid and its salts are likely not skin sensitisers. In the absence of data, ammonium propionate should be treated as propionic acid. No concerns for the environment are expected from the use of those additives up to the recommend use levels. Propionic acid, sodium, calcium and ammonium propionate have the potential to act as preservatives in feedingstuffs. The efficacy of propionic acid and its salts in water was not demonstrated. The use of propionic acid, sodium and ammonium propionate as silage additives did not result in a better preservation of silage. Improved aerobic stability of silage was not sufficiently demonstrated.

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KEY WORDS

Technological additive, preservative, silage additive, propionic acid, sodium propionate, calcium propionate, ammonium propionate

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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 for the target animal(s), consumer, user and the environment and the efficacy of the products propionic acid, sodium propionate, calcium propionate and ammonium propionate.

The applicant applied for the re-evaluation of the use of propionic acid and its sodium, calcium and ammonium salts as preservative to feed, and for a new use of propionic acid, sodium propionate and ammonium propionate in water for drinking and as silage additives for all animal species and categories without restrictions.

Large differences exist in the tolerance to propionic acid between different animal species. Poultry appears to be the most sensitive, followed by pigs; ruminants are rather insensitive. The maximum safe dietary level of propionic acid for poultry is 10 g/kg complete feed and for pigs 30 g/kg complete feed. The corresponding maximum concentrations in water for drinking would be 4 g/L for poultry and 10 g/L for pigs. Ruminants show a high tolerance to propionic acid, the ruminal production being considerably higher than a reasonable feed supplementation. This conclusion is extended to horses and rabbits. The use of propionic acid as a silage additive without a quantitative restriction of propionic acid supplementation would not affect the safety of target animals. Although the information for propionic acid salts is limited, differences in the safety between the salts and propionic acid are not expected.

Propionic acid, sodium propionate and calcium propionate are authorised in the EU for use in food as technological additives. However, ammonium propionate as such is not authorised as food additive in the EU, but it will not essentially behave differently from other propionates in its metabolism. Propionic acid occurs endogenously as a by-product of normal intermediate metabolism. Residues of propionic acid or its salts when ingested by livestock and poultry are expected to be negligible in tissues and products. The use of propionic acid and its calcium, sodium or ammonium salts in animal nutrition is therefore considered of no concern for the safety of consumers.

Propionic acid and sodium propionate are corrosive to the skin and mucous membranes, and strongly corrosive to the eyes. Swallowing the free acid will lead to a strong corrosive effect on mouth and throat and to the danger of perforation of oesophagus and stomach. No data on sensitising effects are available for the acid, but sodium propionate is not a skin sensitiser. Exposure by inhalation should be minimised. In the absence of data, ammonium propionate should be treated as propionic acid.

No concerns for the environment are expected from the use of propionic acid and its salts in animal nutrition at the recommended use levels in animal nutrition.

Propionic acid, sodium propionate, calcium propionate and ammonium propionate have the potential to act as preservatives in feedingstuffs. The efficacy of propionic acid, sodium and ammonium propionate in water has not been demonstrated. However, propionic acid and its salts may protect water (for drinking) against microbial contamination. The FEEDAP Panel noted that the benefit of using propionic acid and its salts in feed may be lost if the use of those additives in water is approved, since the simultaneous use of those additives in feed and water raises concern for the safety of target animals due to the low margin of safety for poultry and pigs.

Propionic acid, sodium and ammonium propionate are used in the ensiling process with the intention of improving silage production and aerobic stability of silages. The use of those additives did not result in a better preservation of ensiled fresh forage. Improved aerobic stability of silage was not sufficiently demonstrated since it could be shown only in two of three studies with easy to ensile materials, and not in the studies with moderately difficult and difficult to ensile materials.

The FEEDAP Panel made some recommendations, particularly relating to the setting of maximum contents in feedingstuffs for use with poultry and pigs.

TABLE OF CONTENTS

Abstract	1
Summary	2
Table of contents	3
Background	4
Terms of reference.....	4
Assessment	7
1. Introduction	7
2. Characterisation.....	7
2.1. Characterisation of the additive	7
2.1.1. Propionic acid.....	7
2.1.2. Sodium propionate	7
2.1.3. Calcium propionate	8
2.1.4. Ammonium propionate.....	8
2.2. Stability and homogeneity	8
2.2.1. Stability of propionic acid and its salts.....	8
2.2.2. Stability in premixtures	9
2.2.3. Stability in feedingstuffs.....	9
2.2.4. Stability in water	9
2.2.5. Homogeneity	9
2.3. Conditions of use.....	9
2.4. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL).....	10
3. Safety.....	10
3.1. Safety for the target species.....	10
3.1.1. Poultry	10
3.1.2. Pigs	11
3.1.3. Ruminants.....	12
3.1.4. Conclusions	12
3.2. Safety for the consumer.....	12
3.3. Safety for the user.....	13
3.4. Safety for the environment	13
4. Efficacy	13
4.1. Preservatives.....	13
4.1.1. In feed.....	13
4.1.2. In water for drinking.....	14
4.2. Silage additive	14
4.3. Conclusions on efficacy	17
5. Post-market monitoring.....	17
Conclusions and recommendations	17
Documentation provided to EFSA	18
References	19
Appendix	21

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; in addition, Article 10(2) of that Regulation 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 a time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the Acids Authorisation Consortium European Economic Interest Grouping (ACIAC EEIG)⁵ for authorisation and re-evaluation of the products propionic acid, sodium propionate, calcium propionate and ammonium propionate, when used as feed additives for all animal species (category: technological additives; functional group: preservative, silage additive) 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 4(1) (authorisation of a feed additive or new use of a feed additive) and under Article 10(2) (re-evaluation of an authorised 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 8 April 2011.

The additives consist of pure propionic acid, sodium propionate, calcium propionate and a preparation of propionic acid partially neutralised with ammonia. These products are permanently authorised for use in all animal species and categories without a time limit and without maximum levels.

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 animal(s), consumer, user and the environment and the efficacy of the products propionic acid, sodium propionate, calcium propionate and ammonium propionate, when used under the conditions described in Table 1.

⁴ OJ L 268, 18.10.2003, p. 29.

⁵ Acids Authorisation Consortium European Economic Interest Grouping (ACIAC EEIG), Avenue Louise, 130A, 1050, Brussels, Belgium.

⁶ EFSA Dossier reference: FAD-2010-0356.

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

Additives	Propionic acid, Sodium propionate, Calcium propionate, Ammonium propionate
Registration number/EC No/No (if appropriate)	E280, E281, E282, E284
Category of additive	1. Technological additives
Functional group(s) of additive	a. Preservatives, k. Silage additives

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Propionic acid – E280	CH₃CH₂COOH	Min. 99.5%	HPLC method (validated and verified)
Sodium propionate – E281	CH₃CH₂COONa	Min. 98.5%	
Calcium propionate – E282	(CH₃CH₂COO)₂Ca	Min. 98.0%	
Ammonium propionate – E284	CH₃CH₂COONH₄ (neutralised propionic acid solution)	Min. 19.0%	

Trade name (if appropriate)	Not appropriate
Name of the holder of authorisation (if appropriate)	Not appropriate

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		Mg or Units of activity or CFU kg ⁻¹ of complete feedingstuffs, supplementary feed (based on end feed) and in water*		
Propionic acid – E280 – All animal species and categories	-	-	See appendix to EC Annex I for detailed proposed conditions of use	-
Sodium propionate – E281 – All animal species and categories	-	-		-
Calcium propionate – E282 – All animal species and categories	-	-		-
Ammonium propionate – E284 – 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 or water (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

This scientific opinion is based in part on data provided by a consortium of companies involved in the production/distribution of propionic acid (E280), sodium propionate (E281), calcium propionate (E282) and ammonium propionate (E284). The FEEDAP Panel has sought to use the data provided together with data from other sources to deliver an opinion and to produce recommendations for the authorisation which would secure the safety of future uses of propionic acid (E280), sodium propionate (E281), calcium propionate (E282) and ammonium propionate (E284) as feed additive.

1. Introduction

Propionic acid (E280) and its salts sodium propionate (E281), calcium propionate (E282) and ammonium propionate (E284) are included in the European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. They are authorised without a time limit in application of Article 9t (b) of Council Directive 70/524/EEC⁷ concerning additives in feedingstuffs (2004/C 50/01) for their use in all animal species as technological additives. No maximum levels of propionic acid and its salts in feeds are established in the EU. The applicant asks for the re-evaluation of the use of propionic acid (E280), sodium propionate (E281), calcium propionate (E282) and ammonium propionate (E284) as preservative to feed and for a new use of propionic acid (E280), sodium propionate (E281) and ammonium propionate (E284) (in water for drinking and as silage additives) for all animal species and categories.

Propionic acid (E280), sodium propionate (E281) and calcium propionate (E282) are authorised for use in food (Directive 95/2/EC⁸) as technological additives. They are also listed in the Codex Alimentarius (2010). Ammonium propionate is not authorised in the European Union as food additive.

2. Characterisation

2.1. Characterisation of the additive

2.1.1. Propionic acid

The additive is a liquid containing at least 99.5 % of propionic acid (CAS Number 79-09-4, chemical formula $\text{CH}_3\text{CH}_2\text{COOH}$). Propionic acid is soluble in water and alcohol. The additive is produced by chemical synthesis following two possible synthetic routes, the one starting with ethylene, carbon monoxide and water, the other starting with propionaldehyde, oxidised to propionic acid in air in the presence of a catalyst, both followed by distillation.

The analyses of five batches from four companies showed concentrations of propionic acid > 99.7 %.⁹ The impurities comply with the specifications set by the Commission Directive 2008/84/EC¹⁰ on the specific purity criteria on food additives other than colours and sweeteners.¹¹

2.1.2. Sodium propionate

The additive is a powder containing at least 98.5 % of sodium propionate in the dried matter (CAS Number 137-40-6, chemical formula $\text{CH}_3\text{CH}_2\text{COONa}$) and showing a maximum of 4 % loss on drying. The solubility of sodium propionate in water is of 100 g/L at 25 °C. The additive is produced by reacting an aqueous solution of sodium hydroxide and propionic acid, followed by drying of the salt.

⁷ OJ C 50, 25.2.2004, p. 1.

⁸ OJ No L 61, 18.3.1995, p. 1.

⁹ Technical dossier/Section II/Annex_II_1.

¹⁰ OJ No L 253/1, 20.9.2008, p. 1.

¹¹ Technical dossier/Section II/Annex_II_1.

The analyses of five batches from two companies showed concentrations of sodium propionate > 99.7 %.¹² The impurities¹³ comply with the specifications set by the Commission Directive 2008/84/EC.

The analysis of three batches of the product from one company showed a maximum of 0.36 % of particles smaller than 50 µm.¹⁴

2.1.3. Calcium propionate

The additive is a powder containing at least 98.0 % of calcium propionate (CAS Number 4075-81-4, chemical formula $(\text{CH}_3\text{CH}_2\text{COO})_2\text{Ca}$), and showing a maximum of 6 % loss on drying. Calcium propionate is soluble in water. The additive is produced by reacting calcium oxide, propionic acid and water, followed by drying of the salt.

The analyses of five batches from three companies showed concentrations of calcium propionate > 99.0 %.¹⁵ The impurities comply with the specifications set by the Commission Directive 2008/84/EC, except for iron (72–110 mg/kg in five batches of the additive from one producer).¹⁶

The analysis of three batches of the product from one company showed a range of 1.7–2.8 % of particles smaller than 50 µm.¹⁷

2.1.4. Ammonium propionate

The additive is a liquid described as containing a maximum of 80.0 % of total propionic acid, a minimum of 19 % of ammonium propionate (CAS Number 17496-08-1, chemical formula $\text{CH}_3\text{CH}_2\text{COONH}_4$), and a maximum of 30 % of water. Ammonium propionate is soluble in water. The additive is produced by partial neutralisation of propionic acid with ammonia or ammonium hydroxide solution.

The analyses of five batches from three companies showed a range of concentrations of ammonia of 9.2–13.4 %, and a range of concentrations of total propionic acid of 53.7–65.0 % (8.9–22.0 % free propionic acid).¹⁸

The analysis of three batches from one producer showed concentrations of arsenic, cadmium, and mercury of < 2 µg/L, < 0.2 µg/L, 0.5 µg/L, respectively. One batch showed a concentration of lead of 3 µg/L, while the other two were ≤ 2 µg/L. PCBs and dioxins+PCB showed averages of 0.031 and 0.122 ng/L, respectively.¹⁹

2.2. Stability and homogeneity

2.2.1. Stability of propionic acid and its salts

Three batches each of propionic acid,²⁰ sodium propionate,²¹ calcium propionate²² and ammonium propionate,²³ stored in white sealed flask not protected from light (propionic acid and ammonium propionate) or in polyethylene cans (calcium and sodium propionate), were stored for 36 months in unconditioned rooms. The analyses of the samples showed a full recovery of the acid and its salts after three years.

¹² Technical dossier/Section II/Annex_II_2.

¹³ Technical dossier/Section II/Annex_II_2.

¹⁴ Technical dossier/Section II/Annex_II_5.

¹⁵ Technical dossier/Section II/Annex_II_3.

¹⁶ Technical dossier/Section II/Annex_II_3.

¹⁷ Technical dossier/Section II/Annex_II_5.

¹⁸ Technical dossier/Section II/Annex_II_4.

¹⁹ Technical dossier/Section II/Annex_II_4.

²⁰ Technical dossier/Section II/Annex_II_13.

²¹ Technical dossier/Section II/Annex_II_14.

²² Technical dossier/Section II/Annex_II_15.

²³ Technical dossier/Section II/Annex_II_16.

2.2.2. Stability in premixtures

Organic acids premixtures

The stability of propionic acid in an organic acid premixture (formic acid 64 %, propionic acid 25 % and water 11 %) was studied for 24 months. The analysis of three batches showed an average recovery of 99.7 % propionic acid.²⁴

The stability of ammonium propionate in premixtures was studied when added to four different commercial premixtures of organic acids (containing various amounts of formic and propionic acid and their salts, lactic acid and acetic acid), stored for 12 months in glass bottles, protected from the light, at 25°C. After 12 months, ammonium propionate was fully recovered.²⁵

Vitamin/mineral premixtures

One batch each of both sodium propionate and calcium propionate was mixed with three vitamin/mineral premixtures (for layers, piglets and ruminants) at levels of 1 %, 1 % and 1.5 %, respectively. The three samples of premixtures treated with the two active substances (six samples total), were stored for six months in polyethylene bottles at room temperature. The analysis showed after six months a full recovery of both sodium and calcium propionate.²⁶

2.2.3. Stability in feedingstuffs

Single batches of broiler, piglet and ruminant feeds were formulated as examples of the major applications of propionic acid and its salts in animal production. The inclusion level of propionic acid, sodium propionate, calcium propionate or ammonium propionate was 1 %, in each of the feeds. Both meal and pelleted forms of the broiler and piglet feeds were studied while for the ruminant feed only the pelleted form was monitored. The samples were then stored at room temperature for three months.

After three months of storage, the four active substances were fully recovered. Pelleting resulted in a loss of 9.9 %, 4.7 %, 9.7 % and 7.5 % of propionic acid, sodium propionate, calcium propionate and ammonium propionate, respectively.²⁷

2.2.4. Stability in water

Three batches each of propionic acid, sodium propionate and ammonium propionate were dissolved in tap water at concentrations of 0.5 %. The samples were stored in closed bottles, at room temperature in a lighted room. After 48 h, the three active substances were fully recovered.²⁸

2.2.5. Homogeneity

From the five feeds used for the stability studies, 10 subsamples were used to study the homogenous distribution of the four active substances. The analyses showed coefficients of variation of 2.3 %, 2.3 %, 3.2 % and 2.9 % for propionic acid, sodium propionate, calcium propionate and ammonium propionate, respectively.²⁹

2.3. Conditions of use

Propionic acid, sodium propionate, calcium propionate and ammonium propionate are intended to be used as preservatives in feedingstuffs for all animal species and categories with no minimum and maximum level. Propionic acid, sodium propionate, and ammonium propionate are also intended to be used in water for drinking and as silage additives, for all species and categories with no minimum and

²⁴ Technical dossier/Section II/Annex_II_18.

²⁵ Technical dossier/Section II/Annex_II_18.

²⁶ Technical dossier/Section II/Annex_II_17.

²⁷ Technical dossier/Section II/Annex_II_17.

²⁸ Technical dossier/Section II/Annex_II_17.

²⁹ Technical dossier/Section II/Annex_II_17.

maximum level. However, the applicant introduced ‘typical levels of inclusion’ in feed, water for drinking and silage as shown in Table 2.

Table 2: Typical level of inclusion in feed, water for drinking and silage of propionic acid and its salts, as proposed by the applicant

Additive	Use in feed (g/kg)	Use in water (g/L)	Use in silage (g/kg)
Propionic acid	0.1–40	0.1–5	1–10
Sodium propionate	0.1–20	0.1–6	1–10
Calcium propionate	0.1–20	-	-
Ammonium propionate	0.1–40	0.1–6	1–10

2.4. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of propionic acid, sodium propionate, calcium propionate and ammonium propionate in animal feed. The Executive Summary of the EURL report can be found in the Appendix.

3. Safety

3.1. Safety for the target species

Regulation (EC) No 429/2008 indicates that for feed additives already authorised for use in food, tolerance studies may not be required in the target species if, based on metabolic body weight ($\text{mg/kg}^{0.75}$), the exposure of animals by supplemented feed is not considerably higher than that of humans by the use of supplemented food.

The exposure of major animal species calculated with a use level of 20 g propionic acid/kg complete feed, ranges from 476 $\text{mg/kg}^{0.75}$ for salmonids to 1427 for chickens for fattening and laying hens, to 2115 for piglets, and to 3107 $\text{mg/kg}^{0.75}$ for dairy cows. An average 60 kg human adult would have to consume approximately 10 or 67 g of propionic acid daily to achieve use levels equivalent to 476 and 3107 $\text{mg/kg}^{0.75}$, respectively. Assuming that the average adult consumes 1.5 kg of food daily, daily intakes of 10 or 67 g propionic acid would be equivalent to approximately dietary levels of 6700 and 45000 mg/kg food, respectively. Taking into consideration the permitted food additive uses of propionic acid in the EU (the highest maximum permitted concentration is 3000 mg/kg), dietary concentrations of propionic acid ranging from 6700 to 45000 mg/kg food could not be attained.

It is therefore concluded, that the exposure of animals from the anticipated use of propionic acid in animal nutrition (typical high inclusion level: 20 g propionic acid/kg feedingstuffs) as preservative in feed, water for drinking or in silage would be considerably higher than that of humans by the use in food.

Animal data showing safe/tolerated use levels of propionic acid are therefore required. Specific tolerance studies were not provided by the applicant.

3.1.1. Poultry

Numerous trials were published for poultry species and categories. It should be noted that the primary objective(s) of the majority of these studies was not to evaluate the tolerance to propionic acid and/or its calcium, sodium, or ammonium salts.

Cave (1984) found in a 28-day experiment with chickens for fattening, supplementing graded levels of propionic acid (0, 10, 20, 30, 50, 70 and 100 g/kg complete diet) a dose dependent depression of feed intake, starting at 10 g/kg and reaching significance at 50g/kg, weight gain was numerically reduced at

20g/kg. In a second experiment, the same author compared the effects of propionic acid in feed and water for drinking. A significant depression of feed intake and of body weight gain after 28 days was observed with 50 g propionic acid/kg feed (the lowest dose tested) and to a similar extent with 20g/L water for drinking. Also 6.7 g/L resulted in a significant reduction of feed intake and body weight gain. Pinchasov and Elmaliah (1994) fed female broiler chicks (seven days old) for 14 days with diets of different metabolisable energy (ME) contents supplemented with 0, 10 and 30 g propionic acid per kg complete feed. Feed intake decreased significantly in a dose dependent manner with the inclusion of the acid. Also body weight and body weight gain decreased considerably with acid supplementation. Gain to feed ratio was not affected.

In contrast to these results, Vogt et al. (1981) could not observe any negative effect of propionic acid at levels of 5, 10 and 20 g/kg diet and of Ca-propionate at 25.5 g/kg in a six-week study on chickens for fattening. Also no significant negative effects of the same levels of propionic acid were found by the same authors in a 322-day study on laying hens concerning laying performance, egg weight and feed to egg mass ratio, however egg shell thickness was significantly reduced at 10 and 20 g propionic acid/kg feed.

Several studies evaluating propionic acid at dietary levels of 10 g/kg or less demonstrated that no adverse effects on performance or other parameters should be expected (Oruwari (1993); Huff *et al.*, (1994); Khosravi et al., (2008)). Propionic acid or calcium propionate at inclusion rates of 0.5 or 1.0 g/kg diet did not adversely affect the performance of laying hens in a 28-week study or of broiler chickens in a four-week study (Oruwari, 1993). The maximum tolerable level of propionate in broiler chickens was given with 2 g/kg diet. In a 42-day study, broiler chicks fed a diet containing 2 g/kg of propionic acid gained significantly more body weight than chicks fed a control diet (Khosravi et al., 2008). Growth inhibition was not observed in experiments where in broiler chicks were provided diets containing 4.54 or 9.07 g/kg of propionic acid or calcium propionate from hatch to 6 weeks of age (Huff et al., 1994).

Several studies supporting the safety of calcium propionate supplementation at levels ranging from 1.5 to 8 g/kg diet were published. In a six-week study, dietary inclusion of calcium propionate at 2.5 or 5.0 g/kg did not negatively affect the performance of broiler chickens (Bintvihok and Kositcharoenkul, 2006). The performance of laying hens was not adversely affected by calcium propionate supplementation at levels ranging from 1 to 8 g/kg diet for a period of six weeks (Jensen and Chang, 1976). An eight-week study in broiler chicks demonstrated that calcium propionate at an inclusion rate of 1.5 g/kg diet had a stimulatory effect on the performance of chickens (Senani et al., 1997). No remarkable effect on live weight at 8 weeks, feed to gain ratio, or incidence of mortality were observed in broiler chickens fed diets containing 3.0 to 3.4 g ammonium propionate/kg complete feed or 4.0 g calcium propionate/kg complete feed (Kaczmarek et al., 1985).

In a study involving newly hatched turkey poults, the inclusion of calcium propionate at a level of 40 g/kg in a starter feed or 20 g/L in water for drinking reduced feed intake and significantly decreased body weight after a period of 48 hours (Donaldson et al., 1994).

3.1.2. Pigs

Dietary supplementation with propionic acid at levels of 10 to 30 g/kg for a period of 6 weeks did not significantly influence the growth of piglets while 2 % propionic acid in growing pigs improved daily weight gain significantly (Kirchgessner and Roth, 1982). In another study (Bowland, 1972), the performance of growing pigs was not significantly affected when propionic acid was included at dietary levels of 10 or 40 g/kg feed. Partanen (2001) conducted a meta-analysis of data published from 1970 to 2001 and concluded that propionic acid at dietary levels of 5 to 25 g/kg had a positive effect on the performance of fattening pigs.

In a 12-week dietary study, pigs were fed diets, based either on barley-soybean or barley-canola meal, supplemented with 0 (control), 30, 60 or 90 g/kg of propionic acid (Thacker and Bowland, 1981). Propionic acid at dietary levels greater than 30 g/kg significantly reduced the performance of pigs. No significant effects with respect to serum chemistry were observed.

3.1.3. Ruminants

Ruminants produce large amounts of volatile fatty acids (VFA) by fermentation of carbohydrates in the rumen. Ruminal VFA concentrations are highly variable; they are dependent on the composition of the diet, the quantity of feed, and the time after feeding.

The amount of propionic acid produced during ruminal fermentation of feed was estimated by the FEEDAP Panel to be about 100 g propionic acid/per kg feed DM. From in situ experiments with cattle, the range could be given with 61 to 196 g/kg feed DM (Bauman et al., 1971; Sharp et al., 1982).

Thus, no major health problems, such as rumen fermentation disorders or rumen acidosis, were expected from 'typical inclusion levels'.

3.1.4. Conclusions

Large differences exist in the tolerance to propionic acid between different animal species. Poultry appears to be the most sensitive, followed by pigs; ruminants are rather insensitive.

In poultry, a safe dietary level of propionic acid can be given with 10 g/kg complete feed. The low tolerance to propionic acid (margin of safety probably only 1) suggests introducing a maximum content of propionic acid in poultry complete diets of 10 g/kg regarding animal safety. The corresponding maximum concentration in water for drinking would be 4 g/L.

In pigs, a safe level of propionic acid can be established with 30 g/kg complete diet. Since this level does also not provide a margin of safety greater than 1, the introduction of a maximum content in pig feed with 30 g/kg complete feed is highly recommended. The corresponding maximum concentration in water for drinking would be 10 g/L.

Ruminants show a high tolerance to propionic acid, the ruminal production being considerably higher than a reasonable feed supplementation (i.e. maximum recommended 40 g/kg feed DM). No maximum content is considered necessary. This conclusion can be extended to horses and rabbits.

Also the use of propionic acid as a silage additive does not require a quantitative restriction of propionic acid supplementation.

Although the database for propionic acid salts (calcium-propionate, sodium-propionate, ammonium-propionate) is limited, differences in the safety of these salts and compared to propionic acid are not expected.

3.2. Safety for the consumer

Propionic acid occurs endogenously as a by-product of normal intermediate metabolism. Propionic acid is generated during the oxidation of odd-number fatty acids and the catabolism of some amino acids. The resulting propionyl-CoA is further metabolised *via* methylmalonyl CoA to succinyl CoA, which is an intermediate of the tricarboxylic acid cycle. Therefore, propionic acid and its salts are efficiently metabolised in the organism by entering different metabolic pathways, mainly the fatty acid and tricarboxylic acid pathways.

When propionic acid (or its salts) is ingested by livestock and poultry, residues in meat, milk, or eggs are considered negligible given that propionic acid is used by most organs and tissues and can be metabolised to carbohydrates, amino acids, and lipids.

Propionic acid (E280), sodium propionate (E281) and calcium propionate (E282) are authorised for use in food (Directive 95/2/EC) as technological additives. However, ammonium propionate as such is not authorised as food additive in the EU, but it will not essentially behave different from other propionates. An ADI is not specified for propionic acid (JECFA, 1974).

The use of propionic acid and its calcium, sodium or ammonium salts in animal nutrition is therefore considered of no concern for the safety of consumers.

3.3. Safety for the user

No specific studies were provided by the applicant. However, significant reviews are prepared in the context of cooperation between the International Programme on Chemical Safety and the Commission of the European Communities (IPCS-ICSC) for propionic acid under ICSC: 0806 (IPCS_ICSC, 2005a) and for sodium propionate under ICSC: 0557 (IPCS_ICSC, 2005b). The comments in both documents on the types of hazard, acute hazards/symptoms, preventions and first aid are reflected in the MSDS provided for propionic acid,³⁰ sodium,³¹ calcium³² and ammonium propionate.³³ The following descriptions are based on the MSDS.

Propionic acid is corrosive to skin and mucous membranes, and strongly corrosive to the eyes. Swallowing will lead to a strong corrosive effect on mouth and throat and to the danger of perforation of esophagus and stomach. No data on sensitising effects are available. Inhalation should be avoided.

Sodium propionate is corrosive to skin, mucous membranes and the eyes. The dermal LD50 in the guinea pig is 4960–9930 mg/kg bw. Skin sensitising effects in animals were not observed and it was not sensitising in the guinea pig maximisation test. The LC50 after 4 hours inhalation in the rat was > 4.9 mg/L. Inhalation should be avoided.

In the absence of data it would seem prudent to suggest the same properties for calcium propionate as known for the sodium salt.

Information in the MSDS for ammonium propionate is rather ill defined ‘Contact with skin may cause irritation, erythema, dryness and chapped skin. Ingestion may cause health problems, including stomach pain and sting, nausea and sickness. Vapor inhalation may moderately irritate the lower and upper respiratory tract and cause cough and respiratory disorders’. Considering the content of about 10–20 % free propionic acid in ammonium propionate (see Section 2.1.4.) it appears prudent to suggest the same properties as known for propionic acid.

3.4. Safety for the environment

Propionic acid and its salts are present in the environment. Propionic acid occurs endogenously as a by-product of normal intermediate metabolism. Propionic acid is generated during the oxidation of odd-number fatty acids and the catabolism of some amino acids. Propionic acid and its salts are efficiently metabolised in the organism, mainly by the fatty acid and tricarboxylic acid pathways.

The FEEDAP Panel concludes that there are no concerns for the environment resulting from the use of propionic acid and its salts at typical use levels in animal nutrition.

4. Efficacy

4.1. Preservatives

4.1.1. In feed

Propionic acid (E280) and its salts sodium propionate (E281) and calcium propionate (E282) are authorised for use in food as antimicrobial preservatives. Since the function requested for feed is the same as that used in food, no further demonstration of efficacy is required (Regulation (EC) 429/2008; Annex III, 8.4). A comprehensive literature exists demonstrating the long history of use and showing a preservative action of propionic acid and its salts (i.e. in wet grain, fish meal, tapioca and poultry feed

³⁰ Technical dossier/Section III/Annex III_1.

³¹ Technical dossier/Section III/Annex III_2.

³² Technical dossier/Section III/Annex III_3.

³³ Technical dossier/Section III/Annex III_4.

(Singh-Verma, 1973; Goering and Gordon, 1973; Rahnema and Neal, 1994), and in pig feed (Müller *et al.*, 1985). Matsuda *et al.* (1994) published an extended list of minimum inhibitory concentrations of propionic acid against several strains of bacteria, yeasts, and moulds.

In general, ammonium propionate is not authorised as food additive (unlike the ammonium salt of acetic acid) and would therefore require demonstration of efficacy as a preservative in feed. The additive ammonium propionate contains about 10–20 % free propionic acid and about 40–56 % ammoniated propionic acid (from 50 to 70 % ammonium propionate). It is reasonable to assume that the ammonium propionate would dissociate to propionic acid in the same manner as sodium or calcium propionate. It is therefore concluded that ammonium propionate is equally effective as preservative in feed as sodium or calcium propionate.

4.1.2. In water for drinking

The applicant provided some literature directly or indirectly (effects in animals) supporting efficacy when administered via water.

The applicant concludes that the same effect as observed in feed “may be reasonably expected to be observed based on the MIC values reported by Matsuda *et al.* (1994) against several strains of bacteria, yeasts, and moulds. Mroz (2005) reviewed the antimicrobial potency of short chain fatty acids in pigs (citing Partanen (2001) for the performance responses). He concluded that acidifiers may be administered via feed or drinking water ‘for a specifically targeted pathogen(s) during particular production cycles’. Chaveerach *et al.* (2002) concluded that routine application of organic acids to the water supply on poultry farms could prevent or diminish *Campylobacter* transmission.

Walsh *et al.* (2007) could show the potential for the individual use of dietary and water acidification (2.58 mL of a propionic acid blend (also containing acetic and benzoic acid)/L) to improve nursery pig growth performance in antibiotic-free diets and may further improve pig performance when pigs are being fed an antibiotic. However, no changes in *E. coli* shedding or faecal pH were observed. The author also noted that depressed feed intakes observed when diet and water acidification were combined may be the result of decreased palatability arising from high levels of acid. They mentioned that previous research from the same author (Walsh *et al.*, 2004) also provides evidence to support this claim whereby feed intake was reduced when dietary acidifiers (organic and inorganic acid-based blends) were included at 0.6 % in weanling pig diets.

4.2. Silage additive

Propionic acid, sodium propionate, ammonium propionate

A total of five laboratory experiments each lasting 90 days, made with five different forage samples, was conducted. Each of the five studies used 1.5 L mini silos. All had the capacity to vent gas. In each case, the different batches of the forages were treated with 4.0 L propionic acid/t forage, 5.5 kg sodium propionate/t forage and 6.5 L ammonium propionate/t forage. Forage of the control silos was added an equal volume of water without the additive. Ambient temperature was controlled at 20 ± 2 °C.

The five studies involved a range of forages of differing botanical origin and water-soluble carbohydrate (WSC) content primarily selected to show a wide range of dry matter content (see Table 3). The samples represented material easy to ensile (experiments 1 and 2, and 3), moderately difficult to ensile (experiment 4) and difficult to ensile material (experiment 5) as defined in Regulation (EC) No 429/2008 (Table 3).

Table 3: Characteristics of the forage samples used in the ensiling experiments

Study	Test material	Dry matter content (% fresh material)	WSC ^a content (% fresh material)
1 ³⁴	Grass	39.3	3.6
2 ³⁵	Whole plant corn silage	37.5	3.3
3 ³⁶	Grass	26.7	4.0
4 ³⁷	Grass	31.1	2.0
5 ³⁸	Grass	18.5	1.4

^aWSC: water-soluble carbohydrate.

Replicate silos (three for each treatment) were opened at the end of the experiment and at intermediate interval in all experiments. The content of silos from the end of the experiments were analysed for dry matter content, pH, lactic and volatile fatty acids (VFA) concentration, ethanol, ammonia and total nitrogen. Aerobic stability was also followed by the measurement of the change of temperature after opening. A rise of 3 °C above ambient was taken as indicating a loss of aerobic stability.

Data were statistically examined by the one-sided Wilcoxon-Kruskal-Wallis non-parametric test.³⁹ The results are summarised for propionic acid in Table 4, for sodium propionate in Table 5 and for ammonium propionate in Table 6.

Table 4: Propionic acid: Summary of the analysis of ensiled material recovered at the end of the experiments and the aerobic stability after opening of the silo

Study	Dose L/Tonne	Dry matter loss (%)	pH	Lactic acid (% DM ensiled material)	Acetic acid (% DM ensiled material)	Ethanol (% DM ensiled material)	NH ₃ -N (% of total N)	Aerobic stability (days)
1	0	6.12	4.40	8.13	1.92	0.14	2.78	5.5
	4	6.27	4.43	6.76*	1.66*	0.33*	2.55	7.0*
2	0	5.21	3.90	5.23	0.87	1.03	5.86	2.4
	4	7.08*	4.10*	3.87*	1.55*	2.44*	7.89	8.0*
3	0	9.64	4.03	1.50	1.57	0.42	12.04	5.2
	4	9.29	4.03	1.12*	1.87*	0.11*	12.69	7.0
4	0	5.57	4.10	9.29	2.02	0.19	10.17	7.5
	4	5.23*	4.10	8.37*	2.21*	0.35	9.28	8.0
5	0	7.44	4.53	0.99	0.93	0.81	13.01	7.0
	4	6.09*	4.27*	0.86	1.12	0.51*	10.98	7.0

*: Significantly different from the control value at $P \leq 0.05$.

Propionic acid at a dose of 4 mL/kg fresh material significantly reduced pH value after 90 days and dry matter loss in difficult to ensile material (study 5), whereas it increased both parameters in one of the three easy to ensile materials (study 2). Lactic acid was significantly lower in all easy to ensile materials (studies 1, 2 and 3) and in the moderately difficult to ensile forage (study 4). Acetic acid was reduced in study 1, but increased in studies 2 and 3 (all studies with easy to ensile forages) and study 4 (moderately difficult to ensile material). Aerobic stability was significantly improved in two of three studies with easy to ensile forages but not influenced in the studies with moderately difficult and difficult to ensile material.

³⁴ Technical dossier/Section IV/Annex IV_4.

³⁵ Technical dossier/Section IV/Annex IV_6.

³⁶ Technical dossier/Section IV/Annex IV_8.

³⁷ Technical dossier/Section IV/Annex IV_5.

³⁸ Technical dossier/Section IV/Annex IV_7.

³⁹ Technical dossier/Supplementary Information_October_2011.

Table 5: Sodium propionate: Summary of the analysis of ensiled material recovered at the end of the experiments and the aerobic stability after opening of the silo

Study	Dose kg/Tonne	Dry matter loss (%)	pH	Lactic acid (% DM ensiled material)	Acetic acid (% DM ensiled material)	Ethanol (% DM ensiled material)	NH ₃ -N (% of total N)	Aerobic stability (days)
1	0	6.12	4.40	8.13	1.92	0.14	2.78	5.5
	6.5	6.59	4.60*	7.56*	1.98	0.56*	3.15	8.0*
2	0	5.21	3.90	5.23	0.87	1.03	5.86	2.4
	6.5	5.93*	4.13*	5.97*	1.16*	1.30*	8.17	8.0*
3	0	9.64	4.03	1.50	1.57	0.42	12.04	5.2
	6.5	9.53	4.13	1.72	1.72	0.24*	11.04	7.0
4	0	5.57	4.10	9.29	2.02	0.19	10.17	7.5
	6.5	5.47	4.30*	9.54*	2.22*	0.56	10.10	8.0
5	0	7.44	4.53	0.99	0.93	0.81	13.01	7.0
	6.5	6.37*	4.57	1.16	0.73*	0.20*	12.19	7.0

*: Significantly different from the control value at $P \leq 0.05$.

Sodium propionate at a dose of 6.5 g/kg fresh material did not reduce the pH in the ensiled materials; a significant increase was observed in three (studies 1, 2, 4) out of five studies. Lactic acid was lower in study 1 (easy to ensile material), but higher in study 2 (easy to ensile material) and 4 (moderately difficult to ensile forage). As no significant differences for ammonia nitrogen could be found, protein degradation was apparently not influenced by the treatment. Aerobic stability was significantly improved in the same studies (1 and 2) in which propionic acid had shown a positive effect.

Table 6: Ammonium propionate: Summary of the analysis of ensiled material recovered at the end of the experiments and the aerobic stability after opening of the silo

Study	Dose L/Tonne	Dry matter loss (%)	pH	Lactic acid (% DM ensiled material)	Acetic acid (% DM ensiled material)	Ethanol (% DM ensiled material)	NH ₃ -N (% of total N)	Aerobic stability (days)
1	0	6.12	4.40	8.13	1.92	0.14	2.78	5.5
	4	6.84*	4.50*	7.46*	1.93	0.54*	3.05	8.0*
2	0	5.21	3.90	5.23	0.87	1.03	5.86	2.4
	4	6.45*	4.17*	5.40	1.37*	1.30	19.6*	8.0*
3	0	9.64	4.03	1.50	1.57	0.42	12.04	5.2
	4	10.42	4.17	1.27	1.71	0.12*	15.65	7.0
4	0	5.57	4.10	9.29	2.02	0.19	10.17	7.5
	4	4.13*	4.20*	9.32	2.25*	0.60	21.50*	8.0
5	0	7.44	4.53	0.99	0.93	0.81	13.01	7.0
	4	6.09	4.53	0.48	1.07	0.77	17.96*	7.0

*: Significantly different from the control value at $P \leq 0.05$.

Ammonium propionate at a dose of 4 mL/kg fresh material increased pH value after 90 days significantly in three (studies 1, 2, 4) out of five studies. Dry matter preservation was significantly reduced in two studies. The only significant difference found for the lactic acid concentration was a reduction in study 1. Acetic acid was increased in two studies (one with easy and one with moderately difficult to ensile forages). The considerably higher amounts of ammonia nitrogen measured in all ensiled materials (reaching significance in two studies) is likely to be a consequence of the ammonium ion of the silage additive. As in the studies with propionic acid and sodium propionate, aerobic stability was improved in two studies with easy to ensile materials.

4.3. Conclusions on efficacy

Propionic acid, sodium propionate, calcium propionate and ammonium propionate have the potential to act as preservatives in feedingstuffs. This conclusion is mainly based on the fact that propionic acid and its sodium and calcium salts are authorised preservatives in food.

The efficacy of propionic acid, sodium and ammonium propionate in water has not been demonstrated. The specific function of preservatives as defined in Regulation (EC) No 1831/2003, Annex I (preservatives: substances or, when applicable, micro-organisms which protect feed against deterioration caused by micro-organisms or their metabolites) is not performed by the additive if used in water for drinking, although propionic acid and its salts may protect water (for drinking) against microbial contamination.

The FEEDAP Panel notes that the benefit of using propionic acid and its salts in feed may be lost if the use of these additives in water is approved, since the simultaneous use of these additives in feed and water raises concern for the safety of target animals due to the low margin of safety for poultry and pigs.

Propionic acid, sodium and ammonium propionate are used in the ensiling process with the intention of improving silage production and aerobic stability of silages. The use of these additives does not result in a better preservation of ensiled fresh forage. Improved aerobic stability of silage is not sufficiently demonstrated since it could be shown only in two out of three studies with easy to ensile materials, and not in the studies with moderately difficult and difficult to ensile materials.

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 Regulation⁴⁰ and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Large differences exist in the tolerance to propionic acid between different animal species. Poultry appears to be the most sensitive, followed by pigs; ruminants are rather insensitive. The maximum safe level of propionic acid for poultry is 10 g/kg complete feed and for pigs 30 g/kg complete feed. The corresponding maximum concentrations in water for drinking would be 4 g/L for poultry and 10 g/L for pigs. Ruminants show a high tolerance to propionic acid, the ruminal production being considerably higher than a reasonable feed supplementation. This conclusion is extended to horses and rabbits. The use of propionic acid as a silage additive without a quantitative restriction of propionic acid supplementation would not affect the safety of target animals. Although the information on propionic acid salts (calcium propionate, sodium propionate, ammonium propionate) is limited, differences in the safety for target animals between the salts and propionic acid are not expected.

Propionic acid (E280), sodium propionate (E281) and calcium propionate (E282) are authorised in the EU for use in food as technological additives. However, ammonium propionate as such is not authorised as food additive in the EU, but it will not essentially behave differently from other propionates in its metabolism. Propionic acid occurs endogenously as a by-product of normal intermediate metabolism. Residues of propionic acid or its salts when ingested by livestock and poultry are expected to be negligible in tissues and products. The use of propionic acid and its calcium, sodium or ammonium salts in animal nutrition is therefore considered of no concern for the safety of consumers.

⁴⁰ OJ L 35, 8.2.2005, p. 1.

Propionic acid and sodium propionate are corrosive to skin and mucous membranes, and strongly corrosive to the eyes. Swallowing the free acid will lead to a strong corrosive effect on mouth and throat and to the danger of perforation of oesophagus and stomach. No data on sensitising effects are available for the acid, but sodium propionate is not a skin sensitiser. Exposure by inhalation should be minimised. In the absence of data, ammonium propionate should be treated as propionic acid.

No concern for the environment is expected from the use of propionic acid and its salts in animal nutrition at the recommended use levels in animal nutrition.

Propionic acid, sodium propionate, calcium propionate and ammonium propionate have the potential to act as preservatives in feedingstuffs.

The efficacy of propionic acid, sodium and ammonium propionate in water has not been demonstrated. However, propionic acid and its salts may protect water (for drinking) against microbial contamination. The FEEDAP Panel notes that the benefit of using propionic acid and its salts in feed may be lost if the use of those additives in water is approved, since the simultaneous use of those additives in feed and water raises concern for the safety of target animals due to the low margin of safety for poultry and pigs.

The use of propionic acid and its sodium and ammonium salts did not result in a better preservation of ensiled fresh forage. Improved aerobic stability of silage is not sufficiently demonstrated since it could be shown only in two out of three studies with easy to ensile materials, and not in the studies with moderately difficult and difficult to ensile materials.

RECOMMENDATIONS

The low tolerance of poultry to propionic acid (margin of safety probably only one) strongly argues for introducing a maximum content of propionic acid and its salts in poultry complete diets of 10 g/kg regarding animal safety. The corresponding maximum concentration in water for drinking would be 4 g/L.

Since the safe level in pigs does not provide either a margin of safety greater than one, the introduction of a maximum content in pig feed with 30 g/kg complete feed is also recommended. The corresponding maximum concentration in water for drinking would be 10 g/L.

The FEEDAP Panel also recommends that the simultaneous use of propionic acid and its salts in feed and water for drinking should not be allowed for poultry and pigs because of safety concerns for these target animals. The above-mentioned safe concentrations in feed and water for drinking for poultry and pigs are not additive.

It is recommended to include under other provisions:

The simultaneous use with other organic acids is contraindicated when the product is used at or near the maximum permitted content.

DOCUMENTATION PROVIDED TO EFSA

1. Propionic acid, sodium propionate, calcium propionate, ammonium propionate for all animal species. November 2010. Submitted by Acids Authorisation Consortium European Economic Interest Grouping (ACIAC EEIG).
2. Propionic acid, sodium propionate, calcium propionate, ammonium propionate for all animal species. Supplementary information. October 2011. Submitted by Acids Authorisation Consortium European Economic Interest Grouping (ACIAC EEIG).

3. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Propionic acid, sodium propionate, calcium propionate and ammonium propionate.
4. Comments from Member States received through the ScienceNet.

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APPENDIX

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for propionic acid, sodium propionate, calcium propionate and ammonium propionate.^{41 42}

In the current application authorisation is sought under articles 4(1) and 10(2) for *propionic acid*,⁴³ *sodium propionate*,²⁴ *calcium propionate*,²⁴ *ammonium propionate*²⁴ and E700,⁴⁴ under the category of "technological additives" functional group 1(a) "preservatives"^{24,25} and 1(k) "silage additives" (except *calcium propionate*)²⁴, according to the classification system of Annex I of Regulation (EC) No 1831/2003. According to the Applicant (FAD-2010-0356)²⁴ *propionic acid* (E280), *sodium propionate* (E281), and *calcium propionate* (E282) have minimum purities of 98 %, while *ammonium propionate* (E284) has a minimum purity of 19 % in a propionic acid matrix. The feed additive E700 related to application FAD-2010-376 is an aqueous solution containing three active substances, namely (1) sodium benzoate at 140 g/kg, (2) propionic acid at 370 g/kg and (3) sodium propionate at 110 g/kg.

Specifically, authorisation is sought by the Applicant²⁴ for the use of the various *feed additives* (propionic acid, sodium propionate, calcium propionate, ammonium propionate) for all animal species and categories. The *feed additives* are intended to be used in *premixtures*, *feedingstuffs*, *water* and *silage* (except *calcium propionate*). The Applicant²⁴ did not propose any minimum or maximum concentration; similarly to what was set in previous regulation. However, the recommended levels are ranging between 0.1 to 6 g/L for *water*, 0.1 to 40 g/kg for *feed* and 1 to 10 g/kg for *silage*.

Specifically, authorisation is sought by the Applicant²⁵ for the use of the *feed additive* E700 for pigs, bovines, poultry, sheep, goats, rabbits and horses. The *feed additive* is intended to be used in *premixtures* and *feedingstuffs*. The Applicant²⁵ proposes a maximum concentration for the *feed additive* of 10000 mg/kg in complete feedingstuffs and a concentration ranging from 3000 to 22000 mg/kg in cereal.

For the quantification of *ammonium propionate* in the *feed additive* the Applicant²⁴ proposed to combine two methods: - a method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV) for the determination of total propionate (expressed as total propionic acid); and - an indirect titration with sulphuric acid and sodium hydroxide for the determination of ammonia. No performance characteristics were provided. However, the EURL recommends for official control to combine titration and HPLC-RI methods for the indirect determination of *ammonium propionate* in the *feed additive*.

For the quantification of *propionic acid*, *sodium propionate*, *calcium propionate* and *ammonium propionate* (expressed as total propionic acid) in *feed additive*, *premixtures*, *feedingstuffs* and *water* Applicant²⁴ proposed a ring-trial validated method based on high performance liquid chromatography with refractive index or UV detection (HPLC-RI/UV). This method does not distinguish between *propionic acid* and its salts. The following performance characteristics were reported for the HPLC-RI method:

- a relative standard deviation for *repeatability* (RSD_t) ranging from 2 to 17 %;
- a relative standard deviation for *reproducibility* (RSD_R) ranging from 7 to 23 %;
- a recovery rate ranging from 98 to 101 %; and

⁴¹ The EURL produced a combined report for the additives propionic acid, sodium propionate, calcium propionate, ammonium propionate and sodium benzoate, propionic acid, sodium propionate.

⁴² The full report is available on the EURL website: <http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-FAD-2010-0356+0376.pdf>

⁴³ FAD-2010-0356.

⁴⁴ FAD-2010-076 (E700).

- a limit of quantification (LOQ) of 0.07 g *propionic acid*/kg *feedingstuffs*.

Based on the performance characteristics presented, the EURL recommends for official control the ring trial validated method based on ion-exclusion HPLC-RI to determine *propionic acid*, *sodium propionate* and *calcium propionate* and *ammonium propionate* (expressed as *total propionic acid*) in *feed additive*, *premixtures*, *feedingstuffs* and *water*.

For the quantification of *propionic acid*, *sodium propionate* and *ammonium propionate* in *silage* the Applicant²⁴ did not provide any analytical method or experimental data. Therefore, the EURL cannot evaluate nor recommend any method for official control to determine *propionic acid*, *sodium propionate* and *ammonium propionate* in *silage*.

E700 is a mixture of three active substances; it contains of *sodium benzoate*, *propionic acid* and *sodium propionate*. Therefore, the characterisation of *E700* is derived from the quantification of (i) benzoate, (ii) total propionate and (iii) total sodium amounts. For the determination of *sodium benzoate* in *E700*, *premixtures* and *feedingstuffs* the Applicant²⁵ proposed a single laboratory validated and further verified method, based on high performance liquid chromatography with UV detection (HPLC-UV). The reported precisions range between 0.4 and 5.4 %. Based on the acceptable performance characteristics presented, the EURL recommends for official control the HPLC-UV method to determine *sodium benzoate* in *E700*, within the concentration range covered by the experimental data. For the determination of *total propionate* in *E700*, the EURL recommends for official control the above mentioned ring trial validated method, based on ion-exclusion HPLC-RI. For the determination of *total sodium* in *E700*, the EURL recommends for official control the internationally agreed EN ISO 6869:2000 method, based on atomic absorption spectrometry.

The EURL does not recommend for official control any methods for the determination of *E700* (mixture of three active substances) in *premixtures* and *feedingstuffs*.

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.