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Directorate C - Scientific Opinions
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Opinion of the Scientific Committee for Animal Nutrition on the use of Kofa®Grain pH5 (a mixture of sodium benzoate, propionic acid and sodium propionate) in feedingstuffs for pigs, cattle for fattening and dairy cows

(adopted on 19 June 2002)

1. BACKGROUND

A request for authorising the mix of sodium benzoate, propionic acid and sodium propionate in pigs, cattle for fattening and dairy cows under the following conditions of use as a preservative of feedingstuffs has been submitted to the European Commission.

Table 1

Additive	Chemical formula, description	Species or category of animal	Maximum Age	Minimum content	Maximum content	Other provisions
				mg/kg complete feedingstuff		
Preservatives						
Kofa®Grain pH5: mixture of: – Sodium benzoate – Propionic acid – Sodium propionate	**Additive composition** Sodium benzoate: 140 g/kg Propionic acid: 370 g/kg Sodium propionate: 110 g/kg Water: 380 g/kg **Active substance** Sodium benzoate, $C_7H_5O_2Na$ Propionic acid, $C_3H_6O_2$ Sodium propionate, $C_3H_5O_2Na$	Pigs	-	2 200	22 000	For the preservation of grain and feedingstuffs having a moisture content in excess of 15%
		Cattle for fattening	-	2 200	22 000	
		Dairy cows	-	2 200	22 000	

A dossier for the product has been prepared by the company owning the product Kofa®Grain pH5, submitted through the national rapporteur (Germany) to the Commission and has been found by the Member States to be in compliance with the

requirements of the Council Directive 87/153/EEC[1] fixing guidelines for the assessment of additives in animal nutrition as amended.

2. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition (SCAN) is requested to answer the following questions:

2.1. The mix of sodium benzoate, propionic acid and sodium propionate, when used in the feedingstuffs for pigs, cattle for fattening and dairy cows is claimed to affect favourably the characteristics of feedingstuffs by exerting a preservative effect. Is the efficacy of this product demonstrated?

2.2. On the basis of the toxicological data, is the use of sodium benzoate, propionic acid and sodium propionate, safe:
- for pigs, cattle for fattening and dairy cows?
- for the consumer, taking into account total dietary exposure?
- for the user (workers' exposure)?

In assessing the safety of the product for the consumer, the Committee should in particular address the following aspects:
- The metabolic fate of sodium benzoate, propionic acid and sodium propionate in pigs, cattle for fattening and dairy cows.
- The presence of residues in animal tissues, and their qualitative and quantitative composition.

2.3. What is the effect of sodium benzoate, propionic acid and sodium propionate under the conditions of use proposed on the microflora of the digestive tract and on the shedding or excretion of pathogenic microorganisms? Is there any risk associated with this?

2.4. What are the nature and the persistence of the excreted products derived from sodium benzoate, propionic acid and sodium propionate? Can these products be prejudicial to the environment?

[1] E.C. OJ n° L 64 of 07/03/1987, p. 19

3. OPINION OF THE COMMITTEE

3.1. Introduction

Many organic acids are recognised as effective preservatives of foods and feeds with a long history of apparent safe use. Their primary mode of action is through pH depression and their effect is essentially bacteriostatic or fungistatic in nature. However, the ability to change from dissociated to undissociated form depending on environmental pH can transform some organic acids into potent bacteriocides and fungicides. It is only in the undissociated form that acids can diffuse through the cell membrane of micro-organisms into the cytoplasm. Subsequent dissociation of the acid as a result of the near neutral pH conditions in the cell results in the disruption of a number of metabolic processes, leading to cell death. Organic acids with a high pK_a are generally the more effective preservatives.

Propionic acid is produced in large quantities in the rumen of ruminants, where it derives ultimately from the microbial breakdown of ingested plant material. Amounts of propionate generated vary depending on the nature of the diet fed, but represent between 20-40% of the total volatile fatty acids (VFAs) with rumen concentrations of between 20-40mM. Total production measured as entry rate varies between 13 – 31 moles/day in the lactating dairy cow and is greatest in diets high in cereal concentrates and less in diets rich in roughage. Propionate is similarly produced by bacterial fermentation in the colon of non-ruminants including the pig and humans. VFAs, including propionic acid, are passively and rapidly absorbed from the digestive tract, particularly when the luminal pH is below the pK_a. Once absorbed, the bulk of propionate is directed to glucogenesis in ruminants and to catabolism in non-ruminant species.

Propionic acid has been used extensively to aid the conservation of cereal grains, forages and mixed animal feeds. Its addition is intended to prevent growth of fungi acquired as contaminants in the field or on storage and the consequent production of mycotoxins and generally to secure feed hygiene. Initial use was made of the free acid, but latterly the sodium or ammonium (buffered propionate) salts have been preferred for safety reasons. Both the free acid and its sodium salt are permitted additives in the EU for the purposes of feed preservation. Organic acids, including propionic acid and its salts, are used also as zootechnical feed additives in animal nutrition, ostensibly as acidification agents, but also because of benefits to reduced morbidity and improved performance characteristics in young animals achieved through modifications to the gut flora (Partanen and Mroz, 1999).

Because of its corrosive nature, the free acid is rarely used in the food industry, the sodium and calcium salts being found preferable. Salts of propionic acid, while effective inhibitors of filamentous fungi, show very limited activity against *Saccharomyces* spp. because of the presence of an effective efflux mechanism (Holyoak *et al*., 1999). For this reason they have found favour in the baking industry where they are used as preservatives to maximum permitted levels of approximately 3 g/kg, although the actual

levels used are generally well below the permitted maximum. Because of their use in bread and other bakery products, propionates are the most extensively consumed of all chemical preservatives. Estimates of daily intake in Japan was 3.88 mg/person (Ishiwata *et al*., 1997) but this would be expected to be higher in countries with a substantially greater bread consumption.

Benzoic acid occurs naturally in a number of human food items (some berry fruits, prunes) and, as the free acid or its sodium, potassium and calcium salts, is a permitted preservative (95/2/EC) for human foods. The sodium salt has been used for this purpose for over 100 years in many countries. The maximum levels permitted vary greatly depending upon application, ranging between 0.2 g/kg in salted dry fish to 5 g/kg in liquid eggs. Maximum values around 1 g/kg are more typical for most applications.

Ruminants excrete large amounts of conjugated benzoic acid in their urine. The glycine conjugate hippuric acid predominates, but lesser amounts of the glucuronide (benzyl glucuronide) and the free acid can be detected. Most of the benzoic acid derives from hydroxycinnamic acids consumed as components of the ingested forage, but some derives from aromatic amino acids and from hydroxycyclohexane carboxylic acids, such as quinic or shikimic acid (Pagella et al., 1997). These are transformed initially by microbial action and then, subsequent to absorption across the rumen wall, further modified by β-oxidation/aromatisation in the liver to give benzoic acid, which is then generally conjugated and excreted. There is little natural exposure to the free acid.

Benzoic acid, whether added to the diet or derived from plant sources, is similarly conjugated and excreted in non-ruminants and in humans. There are, however, some differences in the ratio of conjugates found in the urine depending on dose or amounts of benzoate generated. In virtually all cases hippuric acid formed in the liver dominates and only when the available glycine pool is reduced is the glucuronide formed in the kidney or the free acid detected in anything greater than trace amounts (Scheline, 1991).

3.2. Nature of the additive and proposed use

Kofa®Grain pH5 is a liquid product consisting of sodium benzoate (140 g/kg), propionic acid (370 g/kg) and its sodium salt (110 g/kg) dissolved in water and has a pH within the range 4.8 - 5.2. It is intended for the preservation of cereal grains, and the recommended application rate is 2.2 – 22 kg/tonne depending on the moisture content of the grain and intended period of storage. Given a product density of 1.11 kg/l, this corresponds to a liquid application rate of 2-20 l/tonne. The acids and their salts are described as being of food or feed grade quality and the final product is routinely monitored for contamination by heavy metals (arsenic, lead, cadmium and mercury).

The liquid product is stable at ambient temperatures (15-25^{o}C) for a least a year. Once applied to grain, recoveries of 80-100% have been measured by gas chromatographic methods after storage periods of up to ten months.

The product is intended to control the growth of filamentous fungi and yeast in moist grains during storage and, consequently, remove any risk of mycotoxin production. The greatest antimycotic effect is said to derive from the benzoate component of the mixture and its action to be maximised by the pH lowering effect of the free propionic acid. Treated grain is intended for use in rations for pigs of all categories, cattle for fattening and dairy cows.

3.3. Efficacy of the product

3.3.1. Determination of application rate

A total of six laboratory tests, three with samples of spring barley and three with winter wheat, to determine the effect of moisture content on the minimum effective dose are described. The grain was air-dried and adjusted to 17.5, 20.0, 22.5, and 25% moisture and Kofa®Grain pH5 applied at 0, 3, 5, 7, 9 and 11 l/tonne. The treated grain samples were stored at 25°C and sub-samples examined after 1, 3, 6, 9 and 12 months for evidence of deterioration as judged by the degree of fungal contamination. The results demonstrated that the minimum rate of 2 l/tonne is adequate to stabilise grain only when the moisture content is below 14%. Above this moisture content at least 3 l/tonne is needed even for short periods of storage (1-3 months). This conflicts with the recommended use, which is restricted to grain with a minimum of 15% moisture. With this moisture content a higher concentration than the minimum claimed would be required.

The data from these experiments support an upper application rate of approximately 11 l/tonne for the two cereals (wheat and barley) examined. Application rates above about 11 l/tonne are claimed necessary for maize with a generally higher moisture content at harvest than other cereals.

3.3.2. Small-scale laboratory studies

A number of other small-scale laboratory studies were made with whole wheat or whole barley grains. These were undertaken to demonstrate the need for all three components of the Kofa®Grain pH5 mixture and also to establish effective dose levels. In addition to the Kofa®Grain pH5-treated grain and untreated grain included in all cases, one experiment included grain treated with sodium benzoate alone for comparison purposes, two experiments included propionic acid alone and one experiment a mixture of the sodium salts of benzoic acid and propionic acid. In each case the alternative formulations were less effective than Kofa®Grain pH5. Results for the Kofa®Grain pH5 component of these five experiments are shown in Table 2.

Table 2. Summary of the results from four laboratory-scale experiments with Kofa®Grain pH5.

Grain	Moisture (%)	Dose (l/tonne)	Duration (weeks)	Suppression of fungal growth obtained with:
Wheat	21.4	0, 5, 10	24	5 l or greater
Wheat (2 samples)	17.1, 21.5	0, 5, 10	64	5 l or greater
Wheat	21.0	0, 2, 4, 6	44	6 l
Wheat and barley	27.5, 20.0	0, 3, 5, 7, 9, 11	48	7 l or greater

3.3.3. Production scale experiments

Production scale trials were held in Germany at various locations during 1996 and 1997. All stored material was treated.

(1) Wheat grain (13-17% moisture, 80 tonnes) was stored in three piles for seven months. The calculated dose was 4-5 l/tonne and the measured concentration equivalent to actual doses of 3.9, 5.0 and 6.0 in the three piles. Regular sampling did not show any irregularities and a decreasing temperature was observed all piles. In addition, monitoring of one of three piles showed that the concentration of propionate and benzoate remained constant throughout the storage period.

(2) Ground wheat (19-22% moisture, 200 tonnes) was stored for ten months in silo after treatment with a measured 7l/tonne. In the subsequent season a further 280 tonnes of ground wheat (13-14% moisture) treated in two lots with 7.4 and 8.6 l/tonne respectively was stored in the same silo. No unusual changes in silo temperature were observed and there was no evidence of failure due to fungal contamination in either year.

(3) Rye grain (16-19% moisture, 270 tonnes) was successfully stored for seven months in sheltered piles after treatment in two lots with a calculated 5 l/tonne (actual values 2.7 and 4.6 l/tonne). Again, monitoring of one of the two piles showed that concentration of propionate and benzoate remained essentially constant

(4) Whole wheat grain (13-15% moisture, 100 tonnes) was stored for ten months in a tower silo after treatment in two lots with a metered 5 l/tonne. Subsequent analysis of the treated grain showed that the actual application rates were 5.5 and 7.5 l/tonne. Samples were withdrawn for mycological examination every 14 days and the levels of propionate and benzoate measured after six months and at the end of the storage period. After ten months the concentration of acids was little reduced and the fungal load was considered within the normal and acceptable range.

(5) Ground wheat (13-16% moisture, 100 tonnes) stored in a bunker silo for three months after treatment in two lots with a notional 2-3

l/tonne. However, problems with the meter did not allow the dose to be accurately determined and subsequent analysis showed that the actual application rates were 1.7 and 2.2 l/tonne for the two lots. Higher than ambient temperatures were recorded in the silo for the first two weeks of storage, but thereafter the temperature fell. At the end of the three-month storage period and following a mycological examination, the stored grain was considered to fall within the normal range and to be acceptable for feeding purposes.

(6) A single experiment was made with ten tonnes of maize grain (35% moisture) which was treated in three lots with a target dose 15-18 l/tonne (actual doses 15.8, 17.2, 20.2l/tonne) and stored for nine months. This dose range provided adequate protection against spoilage for all except those grains exposed at the immediate surface.

As none of the above production experiments included any form of control samples in which grain was left untreated, it can only be presumed from experience and the results of the laboratory experiments that the grain samples would have developed significantly more mycological contamination in the absence of treatment. Several factors combine to determine the likelihood of mycological spoilage:

- Moisture content and distribution of moisture
- Ambient temperature and duration of storage
- Degree of infection by spoilage fungi at harvest
- Physical damage to kernels and the degree of infestation by insects and mites.

A guide to the likely maximum storage time of untreated, freshly harvested grain before deterioration becomes evident suggests 14 days for grain of 16% moisture content falling to six days for grain with a 20% moisture content (Weissbach and Schmidt, 1982). In comparison with these guideline periods, grain treated with Kofa® Grain pH5 was stable for far longer periods.

3.3.4. Monitored farm-scale applications

During 1997-1999 approximately 105,000 tonnes of cereal grain were treated with Kofa®Grain pH5. Approximately 10% of farm sites were sampled for microbiological examination (filamentous fungi and yeasts). In only 2% of cases was the treated grain rejected as sub-standard. Further investigation by the Notifier suggested that under-dosing was the most common reason for failure

3.3.5. Mycotoxin production

Randomly selected grain samples (26) from the 1998 harvest taken from 14 farms using Kofa®Grain pH5 as preservative were tested for numbers of spoilage fungi and for aflatoxin B_1 production. All proved negative for the

mycotoxin. In 2000, five additional samples of grain treated on-farm (barley, rye, wheat and triticale) were again examined for aflatoxin B_1 and for evidence of the presence of the *Fusarium*-derived toxins, deoxynivalenol and zearalenone, and for ochratoxin. No evidence of toxin production was detected.

3.3.6. Conclusions

There is adequate evidence that the addition of Kofa®Grain pH5 to freshly harvested wheat, barley, oats, rye and triticale can substantially extend the storage life of the grain by preventing the development of storage fungi (including yeasts) and the consequent deterioration of the grain.

Although there is no reason to suppose that the product would not be efficacious when applied to maize kernels, evidence for this is limited to a single practical experiment and the extrapolation of the linear relationship between required application rate and moisture content established with other cereals. Further data is needed to substantiate this element of the claim.

The evidence provided on the control of storage fungi is supported by a failure to detect aflatoxin in any of the treated samples of grain examined. Examination of grains for contamination with other mycotoxins, either those typical of *Fusarium* spp. or derived from *Penicillium/Apergillus* spp. was more limited. Generally, field fungi do not generally thrive under storage conditions and thus any mycotoxin produced from such sources would tend to reflect the degree of contamination in field rather than a failure of Kofa®Grain pH5 to control post-harvest growth. However, this may not be wholly true for ochratoxin-producing strains where some post-harvest growth may occur.

Practical experience with the product reported by the Notifier suggests that the dose of Kofa®Grain pH5 actually applied may vary significantly from the intended dose. This may be a particular problem with the lower application levels.

3.4. Safety for the target species

3.4.1. Tolerance studies

Pigs. A total of 20 fattening pigs (average live weight 55kg) were assigned to one of four groups. The first was a control group fed a standard commercial diet and the other three were experimental groups fed the same diet but supplemented with 22,000 mg/kg Kofa®Grain pH5 (x1 maximum recommended dose), 55,000 mg/kg Kofa®Grain pH5 (x2.5 maximum recommended dose) or 110,000 mg/kg Kofa®Grain pH5 (x5 maximum recommended dose). The duration of the study was four weeks during which time performance data was recorded and the animals monitored by a veterinarian and metabolic and blood parameters analysed. At the end of the study animals were slaughtered and the condition of the mucosal layer from the pharynx, oesophagus and stomach examined.

No significant effect on feed consumption was recorded. Intake was numerically higher in those animals receiving x1 and x2.5 Kofa®Grain pH5 compared to controls, while those animals receiving the highest dose showed a reduced intake. However daily body weight gain and the ratio of feed to gain were not significantly affected. Pigs remained clinically healthy throughout the trial and no medical interventions were necessary. Blood and metabolic parameters were within normal ranges and were not significantly affected by treatment. Macroscopic examination of the mucosal samples gave no indication of lesions, keratinization or erosion.

Cattle. The tolerance study with young cattle (average body weight 190 kg) followed the same general design and duration as that used for the fattening pigs (*i.e.* 20 animals assigned to one of the four groups for a period of four weeks). However, a six day adaptation period was included before measurements were made and animals were allowed *ad libitum* access to grass silage. The product was added only to the commercial feed, each animal receiving 2 kg feed/day.

No significant effects on intake occurred although the animals receiving the recommended dose (x1) had a numerically higher feed consumption than the animals receiving the untreated feed. This was reflected in the figure for body weight gain where again, although no significant differences were observed, a numerical increase equivalent to a 20% increase on controls was seen in the x1 group. Cattle remained clinically healthy throughout the trial period and showed no signs of rumen or metabolic disorders. Blood parameters were within normal ranges and were not significantly affected by treatment.

3.4.2. Effect on gut flora

A total of four fattening pigs were fed a diet supplemented with the maximum proposed application level (22,000 mg/kg feed) and a further four pigs the same diet without supplementation. After 23 days the animals with an average body weight of 76 kg were slaughtered and samples taken from the stomach, duodenum, ileum and colon. The pH of each sample was determined and counts made of enterobacteria, enterococci, lactobacilli and yeasts. There was a tendency towards lower pH values in the treated group compared to the control group, particularly in the ileum. The lower number of enterobacteriaceae in this section of the gut compared to the control animals (log c.f.u. 3-5.5 compared to log c.f.u. 6.3-8.5) was probably attributable to the lowered pH. Otherwise no changes in counts of the studied flora of any consequence were evident.

No comparable study was made in functioning ruminants. However, it is recognised that the rumen microflora is highly adapted to the presence of high concentration of VFAs, including propionic acid, and that the most likely adverse effect is a failure of saliva to adequately buffer rumen acidity and maintain a pH above 5.5 –6.0. Below this value, numbers of cellulolytic organisms are reduced. The results of the tolerance test with cattle strongly suggest that this did not occur even when a five-fold greater concentration that the maximum claimed was added. Consequently, it is very unlikely that

a more detailed microbiological examination would reveal any effects that might be considered adverse. In addition the benzoic acid component is primarily effective against eukaryotes and has minimum effects on most bacteria, particularly at the concentration that would apply in the rumen when used with an adult ruminant (<150 mg/l).

3.4.3. Conclusions

The product appeared well tolerated by fattening pigs at up to five times the current maximum recommended dose and thus can be considered safe for this target species when applied at concentrations no greater than the maximum recommended dose.

The same conclusion can be reached for fattening cattle, but only for those produced with the relatively low intensity feeding system used in the tolerance study. However, this feeding system is not typical of many parts of Europe. The amount of concentrates used (2kg/ animal/day) is far below that used in more intensive production systems (beef production and high yielding dairy cows). Animals in these production systems may be especially sensitive as they already may have borderline acidosis and a relatively high incidence of liver abscesses. Therefore studies on diets containing high amounts of cereals, which already lead to high propionate production, would be more relevant as they, concomitantly, would lead to a greater exposure to Kofa®Grain pH5.

No data was provided on water intake by animals fed treated grain. However, the additional sodium consumed as part of the product is not expected to cause problems for animals with *ad libitum* access to water. In practice, dairy cows in particular tend to be deficient in sodium, which has to be added to the diet.

No significant effect of the product on the gut flora of the pig was detected and those numerical changes that were recorded would be considered as potentially beneficial. A study of the rumen flora is considered unnecessary in the absence of any effects on performance or rumen function. Similarly, since both propionate and benzoate are essentially fully absorbed through the rumen wall, no effects on the microbial flora would be expected in the post-rumen digestive tract.

No specific studies were made to examine the potential of the treated grain to increase the population size/shedding of human enteropathogens. However, in the absence of any detected changes in the gut flora of the pig and given the minimal effects of benzoic acid on most bacteria, it is very unlikely that the use of Kofa®Grain pH5 would create conditions which would lead to an increase in numbers of *Salmonella* spp or other potentially pathogenic Enterobacteriaceae. Similarly, strains of *E coli*, particularly the highly virulent O157 serotypes, are not tolerant of VFAs in the rumen of cattle and most fail to multiply (Booth *et al*., 1999). Consequently, addition of Kofa®Grain pH5 is more likely to aid the suppression of strains of *E.coli* rather than promote their multiplication in the rumen.

3.5. Safety for the consumer

3.5.1. Chemical safety

As previously indicated (Section 3.1), propionic acid (E280), sodium propionate (E281) and sodium benzoate (E211) are all permitted food additives that may be added directly to food intended for human consumption. Propionic acid and sodium propionate are already permitted additives for use in preserving grain intended for animal use. In addition, sodium propionate is permitted for use in veterinary medicines and is exempted from the need for a maximum residue limit (Annex II of Regulation 2377/90). Sodium benzoate is permitted for use in green fodder silage and is also used at doses of 250-500 mg/kg body weight/day as a human medicine to treat urea cycle enzymopathies.

The Joint WHO/FAO Meeting of Experts on Food Additives (JECFA) has set ADIs for propionic & benzoic acids and their salts. The ADI for propionates is "not limited", indicating that it was not considered necessary to set a limit, as the intended use would not leave toxic amounts of propionate in food (17th Meeting of JECFA, 1973: WHO Food Additive Series 5; WHO Technical Report Series 539).

In 1967, the JECFA set a group ADI of 5 mg/kg bw for benzoic acid and its derivatives. In 1994, EC Scientific Committee on Food (SCF) set a temporary ADI of 5 mg/kg bw for benzoic acid and its salts. The SCF requested further studies: an investigation of clastogenicity *in vivo* and a developmental toxicity study (Reports of SCF, 1996, 35th & 36th series).

In 1996, the 46th meeting of JECFA reviewed benzoates again (WHO Food Additive Series 37 and WHO Technical Report series 868) and noted that developmental toxicity studies of sodium benzoate were available. Their results were reassuring and consequently the group ADI was kept at 5 mg/kg bw.

In 2000, benzoic acid and its salts were assessed by the WHO's International Programme on Chemical Safety (IPCS), who identified 5 mg/kg bw/day as a provisional tolerable intake of benzoic acid and sodium benzoate (IPCS, 2000, CICAD 26). This limit was provisional because there were concerns over the inconsistent results from *in vivo* studies of the mutagenicity of benzoates. Sodium benzoate had been tested in two bone marrow micronucleus assays that showed no evidence of mutagenicity and in a dominant lethal assay that gave a positive result for mutagenicity. Thus several different committees have identified the same provisional safety limit of 5 mg/kg bw/day for consumer exposure to benzoic acid.

No experimental data was provided by the Notifier on residues occurring in animal products as a result of the use of Kofa®Grain pH5 treated grain as a feed ingredient. Metabolic data indicate that absorbed propionate is fully metabolised and is used as a nutrient by mammals. Benzoate is transformed mainly through glycine conjugation and rapidly excreted as hippuric acid in all mammals, conjugation to glucuronic acid occurring for very high concentrations of benzoate only (WHO, 1996). Experiments on the

distribution and elimination of benzoate in the rat have shown that no accumulation of benzoate occurs in the body (US FDA, 1973). Therefore it can be anticipated that benzoate transfer to milk or benzoate concentration in the tissues resulting from the consumption of Kofa Grain ® pH5 treated grains would not increase significantly the natural exposure of human consumers to benzoic acid.

3.5.2. Conclusions

Feeding of Kofa®Grain pH5 treated grain to animals would not appreciably increase the exposure of human consumers of animal products to propionate or benzoate or their metabolites above the concentrations that are naturally present. However SCAN considers that residue data for benzoic acid and its metabolites in animals would be of general value to evaluate the contribution of the use of Kofa®Grain pH5 to the ADI established for this compound. This is not the case for propionate as a not limited ADI exists.

3.6. Safety for the users of the product

3.6.1. Potential for exposure

Kofa®Grain pH5 is produced in a contained stirred tank, to which water and propionic acid are first introduced, followed by the salts sodium propionate and sodium benzoate. Consequently there is only potential for exposure to the individual ingredients during manufacture. Users of the product are exposed only to a liquid with an approximate pH of 4.8 – 5.2, but there is a potential for inhalation and dermal exposure during the application of the product to the grain and the subsequent handling of the treated grain during feed preparation.

As all of the components of the final product are known and widely used chemicals, with one exception, no new data relevant to the safety of those involved in the manufacture or use of the Kofa®Grain pH5 was generated by the Notifier. The data presented is primarily that summarised in the IUCLID data sheet for each of the three ingredients.

3.6.2. Inhalational toxicity

Values for the inhalation toxicity of propionic acid in rats are LC_{50} of >19.7 mg/l (one hour exposure) and >5.4 mg/l (four hours exposure). No equivalent data exists for the salts of benzoic and propionic acids. However, an LC_{50} of greater than 0.26 mg/l (equal to 260 mg/m^3) was found in rats following exposure to benzoic acid vapour for one hour. The dose of 0.26 mg/l caused lacrimation but no deaths. The vapour pressure of the propionic acid component in the final mixture is considerably reduced compared to the pure compound and the inhalation risk correspondingly reduced. The safety phrase S23 “do not inhale vapour” is included in the Safety Data Sheet and the user recommendations stipulates that the inhalation should be avoided and advises that adequate ventilation should be provided.

3.6.3. Dermal toxicity

A dermal LD_{50} of 500mg/kg body weight in rats and 5-10 mg/kg body weight in guinea pigs for propionic acid has been determined and >10,000 mg/kg body weight in rabbits for benzoic acid. No risk of dermal toxicity for the salts of propionic and benzoic acids has been identified to date.

3.6.4. Irritation of skin and eyes

IUCLID data sheets report the classification of pure propionic acid as corrosive and its sodium salt as "not irritating" to skin. Sodium benzoate was classified as not irritating to skin at doses below 3% but as irritating above this value. It was also found slightly irritating to the eye.

A single acute dermal irritation test, commissioned by the Notifier, was made with three albino rabbits in which the final formulation was applied as a patch for four hours. Animals were examined from one hour to 72hours after removal of the test substance. Slight irritation was observed at one hour but no visible signs of irritation were seen thereafter. Consequently Kofa®Grain pH5 was considered as not irritating to skin. However, as a precautionary measure, the Safety Data Sheet carries the Risk Phrase R36/38 Irritating to eyes and skin.

3.6.5. Sensitisation

Propionic acid and its sodium salt are not considered to have a sensitising potential. However, the IUCLID data includes a number of examples of an adverse reaction to sodium benzoate, particularly amongst those with a history of chronic exposure to benzoic acid or a history of atopic eczema or asthma.

3.6.6. Conclusion

There are no undue risks for those involved in the manufacturer of the product or for those handling the final product, provided note is taken of the risk and safety factors identified in the Safety Data Sheet and the appropriate precautions taken.

3.7. Safety for the environment

As the product is a mixture of natural compounds already present in the environment, no further assessment is necessary. SCAN can conclude that there are no concerns of any significance for the environment.

3.8. Overall conclusion

The product Kofa®Grain pH5 has been shown efficacious for the preservation of cereal grains other than maize when used under the conditions described by the Notifier. However, insufficient data has been presented to determine whether the product is equally efficacious when applied to maize kernels with moisture contents higher than that usually found in the other cereal grains examined.

The product is considered safe for pigs of all ages at the proposed maximum dose and not to result in residues of toxicological concern to consumers of products derived from animals fed the treated grain.

Considering the toxicological data and the metabolic fate of both propionate and benzoic acid, SCAN can conclude that the product Kofa Grain® pH5 is safe for the consumer.

It is considered highly unlikely that use of the product would increase or encourage the multiplication and shedding of human enteropathogens from animals given the treated grain.

Kofa®Grain pH5 should be considered as mildly irritating and the inhalation of its vapour phase should be avoided. Otherwise the product does not pose undue risks to those involved in its manufacture or in handling the final formulation provided appropriate precautions are taken.

Nor is it considered likely that use of the product would have any detectable adverse effects on the environment.

However, while reviewing the data presented by the Notifier a number of other issues were noted by SCAN. These are documented below with accompanying recommendations.

4. RECOMMENDATIONS

In the light of the data provided and the Notifiers decision to restrict the application to cereal grain, the summary of claims made for the product (Table 1) should be amended as follows:

- the moisture content identified (15% or greater) under other provisions should be reduced to 14% or, alternatively, the minimum application level claimed increased (to 3000 to 4000 mg/kg) to be consistent with the experimental data provided.

- the words “and other feedingstuffs” should be deleted.

- until the efficacy of Kofa®Grain pH5 with maize is satisfactorily established, the maximum application rate should be reduced to that necessary to preserve those cereal grains for which adequate evidence of efficacy has been provided (probably 11,000 or 12,000 mg/kg grain). It is also recommended that should use with maize be allowed, the distinction between the maximum application rate needed for cereal grains other than maize and for maize should be retained.

SCAN finds it difficult to see how the use of treated grain could be restricted in practice to the feeding of selected target species. Consequently, it is recommended that additional tolerance and microbiological studies are made with poultry to ensure safety for this category of livestock.

The tolerance study made with fattening cattle provided by the Notifier does not address the major “at risk” group of ruminants; those animals with borderline

acidosis. Consequently SCAN recommends that tolerance studies using high amounts of cereal concentrate (80 - 90 % of DM for growing/fattening bulls and 12-15 kg per day for high yielding dairy cows) are conducted.

SCAN recognises that Kofa®Grain pH5 is intended for the control of organisms leading to the deterioration of stored grain and that its efficacy for this purpose has been demonstrated for grains other than maize. However, SCAN recommends that additional laboratory studies be undertaken to establish the effects of the product at the claimed application rates and in the presence of varying moisture contents on the survival of specific strains of field and storage fungi known to be able to produce mycotoxins. Strains able to produce ochratoxin A should be included.

SCAN was able to conclude on the safety to consumers of Kofa®Grain pH5 without recourse to residue data on benzoic acid because of its extensively documented metabolism. However, residue data for benzoic acid in animals is considered by SCAN to be of general value to risk managers enabling the proportion of the ADI for benzoic acid used by various animal products to be calculated.

5. REFERENCES

5.1. Data provided by the Notifier

Application for the Inclusion of the Formulation "Kofa®Grain pH5" into the Annex of the Council Guideline 70/524/EEC, "Preservatives" Group (April 2000).

First Addendum to the application for the Inclusion of the Formulation "Kofa®Grain pH5" into the Annex of the Council Guideline 70/524/EEC, "Preservatives" Group – Statement on Questions and Remarks made by the Member States with regard to the submitted dossier (July 2000).

Second Addendum to the application for the Inclusion of the Formulation "Kofa®Grain pH5" into the Annex of the Council Guideline 70/524/EEC, "Preservatives" Group – Comments on the questions and remarks by SCAN (submitted on 10.08.2000) on the file which has been presented. (October 2000)

Third Addendum to the application for the Inclusion of the Formulation "Kofa®Grain pH5" into the Annex of the Council Guideline 70/524/EEC, "Preservatives" Group – Comments on the questions and remarks by the member states (submitted up to 28.02.2001) on the file which has been presented and the 1st Addendum (March 2001)

Fourth Addendum to the application for the Inclusion of the Formulation "Kofa®Grain pH5" into the Annex of the Council Guideline 70/524/EEC, "Preservatives" Group – Comments on the questions and remarks by SCAN (submitted on 20.02.2001) on the file which has been presented (May 2001)

5.2. Other references

Booth, I.R., Thomas-Carter, F., Carter, P., Jordan, S., Park, S., Malcolm, L. and Glover, J. 1999. Acid tolerance of *Escherichia coli* – the sting in the tail? In: Escherichia coli *0157 in Farm Animals* (Stewart, C.S. and Flint, H.J. eds.) pp.27-38, CABI Publishing, Wallingford, UK.

Holyoak, C.D., Bracey, D., Piper, P.W., Kucher, K. and Coote, P.J. 1999. The *Scaccharomyces cerevisiae* weak-acid-inducible ABC transporter pdr12 transports fluoresciein and preservative anions from the cytosol by an energy-dep;endent mechanism. *J. Bacteriol.* **181**, 4644-4652.

Ishiwata, h., Nishijima, M., Fukasawa, Y., Ito, Y. and Yamada, T. 1997. Evaluation of preservatives contents in foods and daily intake deduced from the results of the official inspection in Japan in FY 1994. *J. Food Hyg. Soc. Japan* **38**, 145-154.

Pagella, J.H., Chen, X.B., MacLeod, N.A., Orskov, E.R. and Dewey, P.J.S. 1997. Excretion of benzoic acid derivatives in urine of sheep given intraruminal infusions of 3-phenylpropionic and cyclohexanecarboxylic acids. Br. J. Nutr. 77, 577-592.

Partanen, K.H. and Mroz, Z. 1999. Organic acids for performance enhancement in pig diets. *Nutr. Res. Rev.* **12**, 117-145.

Scheline, R.R. 1991. *Handbook of Mammalian Metabolism of Plant Compounds.* CRC Press Inc. Boca Raton USA.

US FDA (1973) Evaluation of the health aspects of benzoic acid and sodium benzoate as food ingredients. Bethesda, MD, US Food and Drug Administration, Life Sciences Research Office (PB-223 837).

Weissbach, F. and Schmidt, L. 1982. *Naturwiss. Reihe*, 31, 89-92.

WHO (1996) Toxicological evaluation of certain fodd additives. Prepared by the 51st meeting of the Join FAO/WHO Expert Committee on Food Additives (JECFA). Geneva, World Health Organization, pp. 403-441, Food Additives Series 42).