EUROPEAN COMMISSION
HEALTH and CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
C2 - Management of scientific committees; scientific co-operation and networks

Opinion of the Scientific Committee on Animal Nutrition on the use of benzoic acid in feedingstuffs for pigs for fattening

(Adopted on 15 November 2002)

1. BACKGROUND

A request for authorising benzoic acid under the following conditions as an acidity regulator of the urine for pigs for fattening has been submitted

Acidity Regulator

EEC No.	Additive	Chemical formula, description	Species or category of animal	Maximum Age	Minimum content	Maximum content
					mg/kg complete feedingstuff	
E 210	Benzoic acid	$C_7H_6O_2$	Pigs for fattening	-	5000	10000

2. TERMS OF REFERENCE

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

2.1. Benzoic acid ($C_7H_6O_2$) (E 210), when used in the feedingstuff for pigs for fattening under the conditions proposed in the above table, is claimed to decrease the pH of the urine together with a reduction in ammonia emission. Is the efficacy of this product demonstrated?

In addition, under these proposed conditions of use, are there any effect on animal production (*e.g.* increased body weight gain, improved feed conversion)?

2.2. Does the use of benzoic acid impair the characteristics of target animals' products?

2.3. On the basis of the toxicological data, is the use of benzoic acid safe:
- for pigs for fattening?
- for the user (workers' exposure)?
- for the consumer, taking into account total dietary exposure?

In assessing the safety of the product for the consumer, the Committee should in particular address the following aspects:
- The metabolic fate of benzoic acid in pigs
- The presence of residues in animal tissues, and their qualitative and quantitative composition

2.4. What is the effect of benzoic acid 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?

2.5. What are the nature and the persistence of the excreted products derived from benzoic acid? Can these products be prejudicial to the environment?

3. EFFICACY

An application has been received requesting the use of benzoic acid, under the trade name VevoVitall, as an acidity regulator of the urine of pigs. The proposed recommended dose range is 5000 to 10000 mg/kg in complete feed.

3.1. Effects on urinary pH and ammonia emission

Studies were performed in pigs to investigate the influence of benzoic acid on
- the pH of urine (6 studies),
- urinary nitrogen (2 studies),
- the pH of slurry (3 studies),
- total nitrogen in DM/excretion (2 studies),
- ammonia emission (calculated in 2 studies; measured in 3 studies) and
- ammonia smell (1 study).

Among the 6 studies on pH of urine, only studies n°1 and 2 appear relevant for the present evaluation, as both are based on the product under evaluation and include the intended level of use. See table 1

Table 1 Influence of benzoic acid (BA) on pH of urine

		Exp. N°						
		1		2	3	5	6	7
		Benzoic acid		BA	BA	Acid mixture including 70% BA	Ca-benzoate	Na-benzoate (sows)
		with fumaric acid	without					
Animals per group		6		20	8	198	5	6
Dosage (%)	0 (control)	7.5	7.5	7.53	7.3	7.5[1]/7.48[2]	6.67[3]/7.18[4]	7.7
	0.2							7.2
	0.3							6.4
	0.7					5.69[1]		5.5
	1	6.5	6.5	6.45				
	1.4					5.02[2]		
	1.5				5.7		5.06[3]/5.68[4]	
	2	5.4	5.4	5.59				
	3	5.1	5.1		5		4.49[3]/5.28[4]	
	4				5			
	5				5			

[1]: starter period
[2]: finisher period
[3]: electrolyte balance : 100 meq/kg DM
[4]: electrolyte balance : 320 meq/kg DM

The pH of urine in control pigs was dependent upon diet and ranged from 6.7 to 7.7 in the various studies. Addition of benzoic acid to the diet reduced the urinary pH of treated pigs. The pH was 6.4-6.6 at 10000 mg/kg of benzoic acid in the diet, it was lower with higher levels of benzoic acid (see table 1). The effect on urine pH at the lowest dose recommended (5000 mg/kg) was not determined.

Table 2 Effect of benzoic acid (BA) on the pH of slurry

		Exp. N°		
		2	5	6
		BA	Acid mixture including 70% BA	Ca-benzoate
Dosage (%)	0	8.18	7.76/7.82	7.87/8.32
	0.7		7.28	
	1	7.76		
	1.4		7.04	
	1.5			6.69/7.08
	2	7.26		
	3			6.26/6.33

Among the studies on pH in slurry, only one study (study 2) is relevant for the present evaluation (table 2). In the other studies, an acid mixture (study 5: 70% benzoic acid, 16,5% acidic calcium salts, 6.5% formic acid and 7% propionic acid) or Ca-benzoate (study 6) were used as additives.

In study 2, pH of slurry decreased from 8.18 to 7.76. This effect and also the effects of higher dosages of benzoate-compounds were lower and less consistent (table 2) than the effect on urinary pH (table 1).

3.2. Effects on animal production

There was no change in feed intake when benzoic acid was added to the feed of pigs at concentrations of up to 15000 mg/kg (Lenis *et al.* 1998b; van Dijk, 1999). However, there was a decrease in feed intake when pigs were fed benzoic acid at a higher concentration of 50000 mg/kg with complete refusal of feed at 100000 mg/kg (Lenis *et al.*, 1998a).

Growth rate of pigs was measured in six studies. Small increases of daily bodyweight gain were observed when dietary concentrations of 5000 to 15000 mg/kg of benzoic acid were used (van der Peet-Schwering *et al.*, 1999; van Dijk, 1999; den Brok *et al.*, 1999). Concentrations of more than 30000 mg/kg of benzoic acid caused significant decreases in daily bodyweight gain (Lenis *et al.*, 1998a). No treatment-related changes in bodyweight gain were observed in other studies of benzoic acid (Lenis *et al.*, 1998b) and of sodium benzoate (Mroz *et al.*, 1998).

3.3. Conclusion on efficacy

Acidification of pig urine can be achieved by use of benzoic acid as a feed additive. However, it is not clear whether the reduction in pH caused by the addition of the recommended doses (5000 to 10000 mg/kg) is sufficient to appreciably reduce ammonia emissions from slurry.

4. IMPACT ON PRODUCTS OF ANIMAL ORIGIN

4.1. Meat and carcass quality

Effects on carcass quality were addressed in two studies.

In the first, (den Brok *et al.*, 1999), 33 pigs (males and females) were fed *ad libitum* with acidified feed, whilst a control group of 33 pigs were given feed without the additive. The acidified feed contained a mixture of organic acids that consisted of 70% benzoic acid (14000 mg/kg in feed), 16.5% calcium acidic salts, 6.5% formic acid and 7% propionic acid. The starter feed contained 1% organic acid mixture (7000 mg/kg benzoic acid); growing/finishing feed contained 2% (14000 mg/kg benzoic acid).

At the start of the experiment the pigs weighed 26 kg on average. At slaughter, the controls weighed 84.1 kg and the test animals 86.7 kg. There was no treatment-related effect on mortality or the general health of the treated animals. Carcasses were assessed for dressing percentage, meat percentage, muscular thickness, fat layer and type judgement. Meat was assessed for pH-warm, pH-cold, drip loss and colour.

There were no differences between controls and test animals with regards to dressing percentage, meat percentage, muscular thickness, fat layer, type judgement, drip loss, colour, pH-warm and pH-cold. However, during the first 24 hrs of storage, the pH of meat from test animals showed a smaller decrease than controls ($P < 0.10$). It was concluded that the use of the organic acids to acidify feeds had no deleterious effect on meat quality.

In the second study (Lenis *et al.*, 1998a), groups of pigs (males and females) were fed diets containing benzoic acid at concentrations of 0 (control), 15000, 30000, 40000 and 50000 mg/kg for 100 days. The pigs initially each weighed within the range 30-40 kg. At the end of the trial, 6-8 pigs from each group were slaughtered. Meat was taken from the lumbar region (3rd, 4th and 5th lumbar vertebrae) of the *M. longissimus dorsi* for meat quality measurements of pH, colour, water holding capacity and intramuscular fat content.

There were some effects on the meat quality parameters measured but all values were claimed to be within normal ranges. There was a dose-related increase in pH of the meat (pH5.5 in controls; pH5.8 at the highest dose). Water holding capacity was significantly decreased ($p<0.05$) at concentrations of 30000 mg/kg or more and colour was affected at the same concentrations. The intramuscular fat content was unaffected. There were no statistically significant differences in any of the meat quality parameters when benzoic acid was added to pig diet at 15000 mg/kg.

4.2. Sensory properties of meat

The sensory properties were tested using meat from the Lenis *et al.*, 1998a study. Triangle difference tests were performed in a trained sensory panel of eight people using cooked cutlets first under white lighting and then under red lights. In these tests panellists were repeatedly presented with three samples of meat, two of which were from the same treatment group to see if the panel could identify the odd one out. Meat from control animals and the different groups given doses of benzoic acid (15000, 30000, 40000 and 50000 mg/kg of diet for 100 days) were compared for flavour, odour, texture, aftertaste and "other characteristics". Differences were noted between controls and all treatment groups.

The triangle tests were followed up with a descriptive test in which eight trained assessors described the sensory characteristics of samples according to an agreed list of characteristics. Meat from treated animals was judged to be more "sour" than control meat and several indicators of texture (less tough, more tender after chewing, less dry after chewing, more smooth/soft) were also altered. These effects were least marked in meat from animals given the highest dose (50000 mg/kg) of benzoic acid. It is unclear how consumers would judge the organoleptic changes that were detected by the taste panellists. The "sour" flavour might be regarded unfavourably but the changes in texture might be regarded as an improvement.

4.3. Conclusion on meat quality and sensory properties

The addition of benzoic acid to pig feed had no effect on the quality of meat from pigs given that feed. Some minor changes to texture, pH and flavour were detected, but it was not clear whether consumers would judge the changes to be favourable or unfavourable.

5. SAFETY OF BENZOIC ACID

5.1. Target species safety

Several studies were performed in pigs and gave results that were relevant to the assessment of the safety to pigs of dietary benzoic acid.

5.1.1. Tolerance

The results of several studies in pigs are summarised in Table 3 hereafter. Inflammatory lesions to the oesophagus and/or the pars oesophagea of the stomach, including hyperplasia, hyperkeratosis, erosions, ulcers and stenosis were seen in three of the studies. In two of these studies there was no indication that these lesions were caused by the dietary inclusion of benzoic acid. However, in the other study, there was a dose-related trend in the severity of the lesions (Lenis *et al.*, 1998a).

Table 3: Tolerance studies

Dietary concentrations of benzoic acid (mg/kg)	Duration of study (days)	Group size	Results	Reference
0, 10000, 20000 and 30000	7	16	No effect on mortality, body weight gain and clinical appearance	Lenis *et al.*, 1998b
0, 10000 and 20000	95	20 (10 males and 10 females)	No effect on gross pathology of lungs, liver, muscle and adipose tissue	Van der Pett-Schwering *et al.*, 1999
0, 15000, 30000, 40000, 50000 and 100000	100	8 (4 males and 4 females)	Liver and kidney weights increase at 40000 mg/kg or more Inflammatory changes to the oesophagus and *pars oesophagea* of the stomach at all treatment levels or with dose-related severity	Lenis *et al.*, 1998a
0, 5000 and 15000	106	64	Lesions to *pars oesophagea* of the stomach in all groups, including controls no dose-related or treatment-related trends	Van Dijk, 1999 Stockhofe-Zuwieden and Wijnands, 1998
0, 10000 and 20000	42	8	Lesions to pars oesophagea of the stomach in all groups, including controls no dose-related or treatment-related trends	Jansman and van Diepen, 2002

Several tolerance studies found lesions of the *pars oesophagea* of the stomach in pigs treated with benzoic acid and in control pigs. In the study by Lenis *et al.*, 1998a, the effect appeared to be more severe and dose-related in pigs treated with benzoic acid. However other authors found no evidence that the lesion was treatment-related. The SCAN is of the opinion that the finding of Lenis *et al.*, 1998a, may have been fortuitous and furthermore, the effects were only seen at doses of benzoic acid that were greater than those requested by the Company. The SCAN concludes that the product is therefore well tolerated by pigs when added to their diet at the level of 5000-10000 mg/kg.

5.1.2. Mineral Homeostasis

Water intake and urine excretion were measured in one study (Mroz *et al.*, 1998). Groups of six pregnant sows were given sodium benzoate in their feed at concentrations of 0 (control), 2000, 4000 and 8000 mg/kg. There were dose-related decreases in both water intake and urine volume (see Table 4). There were dose-related increases in urinary nitrogen, calcium, urea and phosphorus.

Table 4: Water intake and urine volume

Dietary concentration of sodium benzoate (mg/kg)	Water intake (litre/day)	Urine volume (litre/day)
0	11.2	8.9
2000	10.5	8.2
4000	9.0	7.1
8000	5.6	5.6

Following the study by Mroz *et al.* (1998), a study was performed to determine the effect of dietary benzoic acid on the digestibility, urinary excretion and retention of minerals (Sauer *et al.*, 2002). Eighteen castrated male pigs (breed not reported) of average bodyweight of 26.5 kg were initially fed a control diet for seven days. Thereafter they were divided into three groups of six pigs fed for 21 days on either control diet, or diets containing concentrations of 10000 or 20000 mg/kg of benzoic acid. Samples of blood, urine and faeces were collected periodically throughout the study. At the end of the 21-day treatment period the pigs were killed and dissected. The stomach with contents and the left femur were removed for examination.

All pigs appeared healthy throughout the study. There were no effects on intakes of feed and drinking water or on urinary volume. Faecal weight was slightly reduced in a dose-related manner but the effect was not significant. At a dietary concentration of 10000 mg/kg, there was increased urinary excretion of sodium and chloride and decreased retention of chloride. At 20000 mg/kg there was decreased faecal excretion of calcium, phosphorus, sodium and nitrogen; increased urinary excretion of sodium and chloride; increased digestibility of calcium, phosphorus, sodium and chloride; increased retention of calcium and phosphorus; and decreased retention of chloride. Femur weights were slightly but not significantly increased at both groups given benzoic acid and the chloride content of bone was slightly but significantly ($P<0.05$) decreased for the 20000 mg/kg group only. The ash from bone from the 20000 mg/kg group had significantly decreased chloride and significantly increased magnesium. Serum phosphate and pH were significantly increased in the 20000 mg/kg group in blood taken at the end of the study (day 21), but not in blood samples taken at the beginning (day 1) and in the middle of the treatment period (day 10). Urinary pH was significantly decreased in a dose-related manner in both groups given benzoic acid.

Dietary levels of 10000 mg/kg to 20000 mg/kg of benzoic acid did not have an adverse effect on the uptake and retention of calcium, phosphorus, magnesium, potassium and nitrogen in pigs. The only effects seen at the 10000 mg/kg dose were decreased urinary pH, increased urinary excretion of sodium and chloride and decreased retention of chloride. At 20000 mg/kg, the retention of calcium and phosphorus was increased and chloride retention was decreased. There was no loss of these minerals from bone. The digestibility of sodium was increased and of chloride decreased in the 20000 mg/kg group.

The feeding of up to 20000 mg/kg of benzoic acid in the diet of pigs had no adverse effect on mineralisation of bones.

5.1.3. Conclusion on target species safety

High concentrations of benzoic acid added to the diet of pigs caused lesion of the pars oesophagea of the stomach and in some cases also of the oesophagus. The lesions included hyperplasia, hyperkeratosis, erosions, ulcers and stenosis, and they were likely to have been caused by prolonged irritation by dietary benzoic acid. However, there was no evidence that dietary concentrations of benzoic acid within the range 5000 to 10000 mg/kg caused such lesions when fed to pigs. Furthermore the feeding of up to 20000 mg/kg of benzoic acid in the diet had no effect on bone mineralisation in pigs. The SCAN concluded that the recommended use of benzoic acid at dietary levels of 5000 - 10000 mg/kg in complete feedingstuff was well tolerated when administered to pigs for fattening.

5.2. User Safety

5.2.1. Occupational Exposure

There is potential for inhalation and dermal exposure to benzoic acid during incorporation into animal feed and whilst handling the treated feed.

The company has proposed that the commercially available form of benzoic acid for use as a feed additive (VevoVitall) would be supplied as flakes having very low dustiness. The manufacturer has claimed the particle size distribution data for VevoVitall (details of how these data were measured were not available) show that 99.6% of the product would be in the form of particles of diameter greater than 0.8 mm, which is too large to be respired into the lungs (van Nispen, 1999; Joosten, 1999).

5.2.2. Inhalation toxicology

1. 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 1 hr. The dose of 0.26 mg/l caused lacrimation but no deaths. (IUCLID, 1996)

2. In a GLP-compliant study, groups of 10 male and 10 female Sprague-Dawley rats were exposed to 0 (control), 25, 250 or 1200 mg/m^3 of benzoic

acid as respirable dust (MMAD = 4.7 μm) for 6 hrs per day, 5 days per week for 4 weeks. Exposure to 25 mg/m^3 did not produce any clinical signs and there was no effect on clinical chemistry, haematology or gross pathology, but some histopathological lesions were evident. At 250 and 1200 mg/m^3, there was upper respiratory tract irritation (both sexes), as indicated by red material around the nares, and decreased absolute and relative kidney weights (females only). At 1200 mg/m^3, one male and one female died, and mean bodyweight gain was decreased in both sexes. Also in the top dose group, there were lower bodyweight gain, decreased platelet count (males and females), decreased absolute and relative liver weights (males only), decreased trachea/lung weight (females only) and all animals exhibited upper respiratory tract irritation. Microscopy revealed a multifocal to generalised pulmonary fibrosis and inflammatory cell infiltrate in animals from all of the dose groups given benzoic acid. As adverse effects were seen at all doses, a NOEL was not identified. (Rop *et al.*, 1981)

5.2.3. Dermal absorption

Benzoic acid has been reported as being partially absorbed across skin in humans and in various animal species (guinea-pigs, mice, rats, dogs, pigs and rhesus monkeys) (IPCS, 2000).

5.2.4. Dermal toxicity

Dermal LD_{50} > 10000 mg/kg bw in rabbits (IUCLID, 1996).

5.2.5. Dermal irritancy

Only minimal irritancy was reported for benzoic acid applied, as a powder or as a paste, in three GLP-compliant tests for dermal irritancy in rabbits (Daamen and van Wijk, 1988). These studies did not conform to current OECD guidelines.

Benzoic acid caused a concentration-dependent increase in ear thickness, in a test for non-immunological contact urticaria (Lahti and Maibach, 1984, as cited in IPCS, 2000 and IUCLID, 1996).

Several irritancy tests have been performed in humans. Most of the studies showed slight to moderate irritation to intact normal skin. More marked irritation occurred when benzoic acid was applied to scarified skin (IUCLID, 1996)

5.2.6. Eye irritancy

Rabbit studies showed benzoic acid powder to be moderately to severely irritating to the eye (IUCLID, 1996; IPCS, 2000).

5.2.7. Sensitisation

Benzoic acid was tested for skin sensitisation in a series of GLP-compliant

assays in animals. In addition, there have been GLP-compliant investigations of allergy to benzoic acid in humans. Full reports of these tests were not provided.

The skin sensitising potential of benzoic acid was tested in several studies in laboratory animals, using various test protocols (maximisation test, Buehler test, ear-swelling test, local lymph node assay) (IUCLID, 1996; IPCS, 2000).

The only positive result in laboratory animals was obtained in an ear-swelling test in guinea pigs. Five animals were challenged by various concentrations (0.2, 1, 5 or 20% in ethanol) of benzoic acid to both sides of the ear lobe and the thickness of the ear was measured at various (unstated) time intervals.

In humans, urticaria, asthma, rhinitis or anaphylaxis have been reported following oral, dermal or inhalation exposure to benzoic acid or sodium benzoate. The symptoms appeared shortly after exposure and disappeared within a few hours. (IUCLID, 1996; IPCS, 2000)

There have been reports of several studies (including patch tests, skin prick tests and oral provocation tests) on small groups of people suffering from urticaria, dermatitis, asthma or Melkersson-Rosenthal syndrome. The results of these studies demonstrated that some atopic individuals react to dermal or oral challenge with benzoic acid or sodium benzoate (IUCLID, 1996; IPCS, 2000). In a study of 2045 patients of a dermatological clinic, only 5 individuals (approximately 0.2%) showed a positive in patch tests (Brasch et al, 1993, as cited in IPCS, 2000), while 34 of 5202 patients (approximately 0.7%) with contact urticaria reacted positively (Broeckx et al, 1987, as cited in IPCS, 2000).

Although full details were not available for any of the studies of sensitisation, some information can be gathered from the reported results of large number of studies. All of the animal studies showed that benzoic acid did not have skin sensitising potential. The results of the human investigations indicated that some people have an allergy to benzoic acid, presumably becoming sensitised from occupational or dietary exposure. However, given the ubiquitous nature of benzoates in foods and the environment, the prevalence of sensitised individuals in the population was low. It would be prudent to regard benzoic acid as a potential sensitiser, whilst recognising that the individual risk is low. Given the low risk, there is no need to label benzoic acid as a skin sensitiser.

5.2.8. Conclusion on user safety

As the rat inhalation study showed adverse effects at all doses tested, it is not possible to identify a level of inhalation exposure that is without risk. Therefore the SCAN recommends that measures should be taken to minimise the production of respirable dust from this product. The SCAN supports the proposal to supply VevoVitall as non-dusting flakes.

It is apparent from the limited reports available that benzoic acid may be irritant to skin and eyes. Appropriate labelling may be required.

The balance of evidence in skin sensitisation indicated minimal risk to operators.

5.3. Consumer safety

Benzoic acid is a permitted food additive (E210) for use as a preservative in human foods.

In 1994, the Scientific Committee for Food (SCF) considered the safety of the use of benzoic acid and its salts (European Commission, 1996). SCF set a temporary group ADI of 0 to 5 mg/kg bw as benzoic acid equivalents, based on a NOEL of 500 mg/kg bw/day taken from long-term and multigeneration studies. Further studies on teratology and mutagenicity were requested and in 2002 the available data were re-evaluated (SCF, 2002). The SCF subsequently set a full group ADI of 0-5 mg/kg bw for benzoic acid and its salts.

The safety of the use as food additives of benzoic acid and various closely related compounds, that are metabolised via the same route, was evaluated in 1996 at the 46th meeting of the Joint FAO/WHO Committee on Food Additives (JECFA) (World Health Organization, 1996; World Health Organization, 1997). JECFA set a group ADI of 0 to 5 mg/kg bw as benzoic acid equivalents for benzoic acid, benzoates (potassium, sodium and calcium), benzaldehyde, benzyl alcohol and benzyl acetate.

5.3.1. Toxicological aspects

The applicant has not provided full reports of the toxicological studies. Instead reference has been made to the reviews performed by SCF and JECFA. In addition, the IUCLID Data Sheet on benzoic acid has been provided, giving brief summaries of each toxicological study.

5.3.1.1. Pharmacokinetics in laboratory animals

The oral administration of radiolabelled benzoic acid to rats, mice and dogs, has shown that it is readily absorbed then excreted mainly in urine as hippuric acid (71-100%), benzoyl glucuronide (0-25%) and benzoic acid (traces-2%) (Bridges *et al.*, 1970). No accumulation of radioactivity in the tissues occurred (IPCS, 2000).

5.3.1.2. Single-dose oral toxicity

Values of 1940-2263 mg/kg bw have been reported for the oral LD_{50} of benzoic acid in the mouse (IPCS, 2000). Values of 1700-3400 mg/kg bw have been reported for the oral LD_{50} of benzoic acid in the rat (IUCLID, 1996; IPCS, 2000). Clinical signs of intoxication included diarrhoea, muscle weakness, tremors, hypoactivity and emaciation. Cats appear to be sensitive to acute poisoning by benzoic acid with toxic effects occurring after a single dose of 450-890 mg/kg bw.

5.3.1.3. Short-term repeat-dose oral toxicity studies

A large number of short-term oral toxicity studies of benzoic acid and of sodium benzoate have been reported in several new published monographs (WHO 1996, IPCS 2000, IUCLID 1996). Most of the studies were not performed to modern standards. Adverse effects in mice, rats and dogs were observed only at high doses, greater than 1000 mg/kg bw/day. There was some evidence of adverse effects in cats at lower doses, and this is probably a result of the known limited ability of cats to conjugate xenobiotics.

5.3.1.4. Long-term toxicity/carcinogenicity studies

Mice: Groups of 25 male and 25 female mice (strain not stated) were given benzoic acid at a dose of 40 mg/kg bw/day, sodium bisulphite at 80 mg/kg bw/day or a mixture of the two for 17 months. Details of the results of the study were not available. (Shtenberg and Ignat'ev, 1970, as cited in World Health Organization, 1996)

A group of 50 male and 50 female albino Swiss mice was given a 20 mg/litre solution of sodium benzoate (99% pure) as their drinking water throughout their lifetime. An untreated control group consisted of 100 male and 100 female mice. The doses were equal to 0 or 5960-6200 mg/kg bw/day. Animals were either allowed to die or were sacrificed when moribund. Necropsies were performed on all of the animals. The following tissues were examined histologically: liver, spleen, kidneys, bladder, thyroid, heart, pancreas, testes, ovaries, brain, nasal turbinates, lungs and any tissues showing gross abnormalities. Treatment had no effect on survival or on the incidence of tumours. Details of non-neoplastic histopathology were unavailable. It was concluded that benzoate was not carcinogenic in mice. (Toth, 1984, as cited in World Health Organization, 1996 and IPCS, 2000)

Rats: In a pre-GLP study, a group of 10 rats (strain not specified) of each sex received benzoic acid at 40 mg/kg bw/day for 18 months. Growth was slightly reduced and the erythrocyte sedimentation rate was increased. No pathological examination was reported. (Shtenberg and Ignat'ev, 1970, as cited in World Health Organization, 1996; IUCLID, 1996)

Groups of 50 male and 52 female Fischer 344 rats, received diets containing 10000 mg/kg (500 mg/kg bw/day) or 20000 mg/kg (1000 mg/kg bw/day) of sodium benzoate for 18-24 months. A control group of 25 males and 43 females received basal diet. Survival was poor in all groups as a result of infections. All surviving animals were killed for autopsy and histopathology at 18-24 months. No adverse treatment-related effects were seen on clinical signs, bodyweight, mortality, tumour types or tumour incidence. (Sodemoto and Enomoto, 1980, as cited in World Health Organization, 1996)

5.3.1.5. Mutagenicity

The petitionner did not provide any specific study on the mutagenic potential of benzoic acid but provided general information publicly available on this

subject. The SCF has recently reviewed the mutagenicity studies performed on benzoic acid and its salts. A wide range of *in vitro* studies have been performed. Some of them (principally cytogenetic analyses) indicated that benzoates had genotoxic potential. However, *in vivo* studies, including cytogenetic analysis, showed no evidence of genotoxicity. The SCF concluded that benzoic acid and its salts did not present a genotoxic hazard to consumers (SCF, 2002). The JECFA had reached the same conclusion (WHO, 1996-1997).

5.3.1.6. Reproduction studies

Full reports were not available for any of the reproduction studies, but other committees have concluded that no adverse effects were seen in mice or rats at doses of up to 550 mg/kg bw/day (SCF, 2002).

5.3.1.7. Developmental toxicity studies

The developmental toxicity of benzoic acid and its salts has been most recently reviewed by the Scientific Committee on Food (SCF, 2002).

Although some teratogenic effects were seen at high doses in the rat, there was no evidence to suggest that benzoic acid, sodium benzoate or benzyl acetate were teratogenic at doses that were not toxic to the mother. Fetotoxicity was observed in rats with a NOAEL of 500 mg/kg bw/day. No adverse effects were seen in the other species tested. The SCF set a group ADI of 5 mg/kg bw for benzoic acid and its salts, by applying an uncertainty factor of 100 to the NOAEL of 500mg/kg bw/day (SCF, 2002).

5.3.2. Metabolism and residues in the pig

Sows given 50 mg/kg bw radiolabelled benzoic acid in their diet excreted about 50% of their radioactivity in the urine over the first 24h. Hippuric acid was the major (85-93%) urinary metabolite with lesser amounts of benzoic acid (7-15%) and traces of benzoyl glucuronide (Bridges *et al.*, 1970).

No study of residues distribution and depletion in pig was available. Nevertheless, limited indications can be obtained from a study where a pig was administered the highest recommended dietary concentration of benzoic acid for 32 days followed by a three-day single oral dose of radiolabelled benzoic acid of 18 g/day (equivalent to 6000 mg/kg feed). It was confirmed that the radioactivity measured in tissues 24h after the last dosage expressed as benzoic acid equivalent was as follows: liver: 1.2, kidney: 1.6, muscle: 0.26 and fat: 2.4 mg/kg.

5.3.3. Human consumers exposure

Benzoic acid is a natural compound present in food commodities. It is also authorised as a food additive.

Considering the SCAN figures for standard human daily consumption, *i.e.* 300

g meat, 100 g liver, 50 g kidney and 50 g fat, and the total residues (benzoic acid and metabolites) found in pig tissues (see 5.3.2) as a worst case assumption, the daily exposure of the consumer would be 0.4 mg. Considering that the administration to the pig of the highest recommended dose (10000 mg/kg instead of 6000) would eventually double this figure, *i.e.* 0.8 mg, this value would represent 0.25% of the ADI.

5.3.4. Conclusions on Consumer Safety

It is noted that benzoic acid is produced endogenously by living organisms and is present at significant concentrations in various plants entering the human diet. Furthermore, benzoic acid is widely used as food additive.

In this context, SCF has recently issued an opinion on the safety of benzoic acid (SCF, 2002). Therefore, for consumers safety considerations, SCAN wishes to adopt SCF position, namely that an ADI can be set at 5 mg/kg bw based on the NOAEL for developmental toxicity (500 mg/kg bw/day).

A residue depletion study has not been submitted. However data on kinetics indicate that benzoic acid is rapidly metabolised and excreted mainly as hippuric acid and does not accumulate. Considering the results of a very limited pharmacokinetic study, it can be estimated that at the highest recommended concentration (10000 mg/kg), the residues of benzoic acid equivalents in tissues would represent 0.25% of the ADI.

6. MICROBIOLOGICAL SAFETY

6.1. Study of the bacteria in faeces of treated pigs

A bacterial examination was performed on faeces of groups of pigs fed 0, 15000, 30000 or 50000 mg benzoic acid per kg feed. Each group consisted of four male and four female crossbred, (GY x NL) x GY, growing-finishing pigs. Faecal samples were collected on day 78 of treatment and counts were made of the numbers of total aerobic and facultatively anaerobic bacteria, enterobacteriaceae, lactobacilli and sulphite-reducing clostridia. Appropriate methods for the specific isolation and enumeration of *Salmonella* were also included but no strains of *Salmonella* were detected.

There were highly statistically significant ($p<0.01$) reductions in the faecal counts of total aerobic and facultatively anaerobic bacteria, enterobacteriaceae and lactobacilli in all benzoic acid treated groups compared with untreated controls (see Table 7). These reductions were dose-related. There was no significant effect on the counts of sulphite-reducing clostridia.

Table 7: Mean (and standard deviation) of Bacterial Counts in Pig Faeces (log cfu/g)

Dietary concentration of benzoic acid (mg/kg)	Total aero-anaerobic bacteria	Enterobacteriaceae	Lactobacilli	Sulphite-reducing clostridia
0 (control)	8.19 (0.59)	5.65 (0.60)	7.99 (0.43)	4.84 (0.50)
15000	6.51** (0.55)	4.46* (1.04)	6.80** (0.42)	4.40 (0.58)
30000	6.25** (0.30)	4.28** (0.62)	6.01** (0.56)	4.68 (1.44)
50000	5.97** (0.31)	3.80** (0.66)	5.22** (0.81)	4.44 (0.88)

* $p < 0.05$
** $p < 0.001$

The results of this study indicate that concentrations of 15000 mg/kg or more added to pig feed can alter the populations of bacteria excreted in faeces (Lenis *et al.*, 1998a).

A semi-quantitative analysis was made of the activity of selected enzymes from colonies isolated from the faeces of the pigs exposed to benzoic acid and compared to colonies isolate from the faeces of untreated pigs. However, the purpose of this study is very unclear. Bacteria whose metabolic processes had been seriously compromised by the presence of benzoic acid would not be able to grow and form colonies and thus could not have been isolated. For those organisms less seriously affected, the absence of benzoic acid in any of the media used for isolation would have allowed the isolation medium to serve as a recovery medium and most adverse effects would have been reversed. Consequently, the results of this experiment do not provide any reliable information on the effects of benzoic acid on the metabolic activities of gut micro-organisms *in vivo*.

6.2. Conclusions on microbiological safety

All of the concentrations of dietary benzoic acid tested altered populations of bacteria in faeces. However, the lowest application rate (15000 mg/kg feed) was still substantially higher that the recommended dose range and the extent to which lower concentrations would affect bacterial numbers is unclear. Effects on enterobacteria would be expected to mitigate against any overgrowth by *E. coli* and would not encourage growth of other enteropathogens such as *Salmonella* spp. Even, the evident resistance of clostridia to benzoic acid which might offer a selective advantage to *Cl. perfringens* at the highest application rate tested, would be unlikely to be of any consequence at the recommended dose range.

7. SAFETY FOR THE ENVIRONMENT

The dossier does not provide the complete information for environmental risk assessment, as required by the guidelines. In particular, data needed for a precise quantification of PEC in various environmental compartments (soil, water, and air) are not available. Nevertheless, on the basis of the characteristics of benzoic acid and of its metabolic products, it is reasonable to state that there are no reasons of concern for the environment. The arguments to justify this statement are described

below.

The benzoic acid fed to farm animals will be excreted mainly as urinary hippuric acid (World Health Organization, 1996; IPCS, 2000; Bridges et al, 1970). Hippuric acid is an endogenous substance that is a normal product of metabolism. Small amounts of benzoates may occur in excreta. The environmental effects of benzoates have been reviewed by IPCS (2000), who concluded that such substances have a low to moderate potential for bioaccumulation, a low to moderate toxicity to aquatic organisms and a low toxicity in the terrestrial compartment. It is therefore not expected that use of benzoic acid as a feed additive will have adverse effects on the environment.

7.1. Toxic effects on non-target organisms

Toxicity of benzoic acid on aquatic organisms is low or very low. The most sensitive organisms are algae (lowest ecotoxicological end point: *Anabaen*a 3h EC50 = 5 mg/L). For all other aquatic organisms, effect levels are around hundreds of mg/L.

No data are available on terrestrial organisms (besides mammals). Nevertheless, a PNEC for the terrestrial environment is proposed in the dossier by extrapolation from aquatic data. The procedure, even if very rough, can be accepted for such a low toxicity chemical.

No toxicity data are available for hippuric acid (the main excretion product), nevertheless, for analogy with other natural carboxylic acids and normal products of metabolism, it is reasonable to suppose that toxicity would be negligible.

7.2. Environmental distribution and fate

Both benzoic and hippuric acids are highly water soluble and poorly volatile. Therefore they have high affinity for the water compartments. Both chemicals are readily degradable in soil and in water.

7.3. Bioaccumulation

For both benzoic acid and hippuric acid the potential for bioaccumulation is considered negligible.

7.4. Environmental concentrations and risk characterisation

A worst case PEC, for both chemicals, has been calculated for soil at local level. It appears far from levels of concern. Starting from PEC soil, for benzoic acid, concentrations in water should be low, orders of magnitude below a toxicological end point for aquatic organisms.

This cannot be stated for hippuric acid, due to the lack of toxicological data, but it can be reasonably hypothesised that adverse effects on the aquatic environment are unlikely to occur.

7.5. Conclusions on environmental safety

The benzoic acid used to treat feed will be excreted primarily as hippuric acid and benzoates and no adverse effects to the environment are expected form the parent compound or its metabolites.

8. OVERALL CONCLUSIONS

8.1. The addition of benzoic acid to pig feed has a dose-related effect on lowering the pH of urine, which in turn is supposed to decrease the ammonia emission from slurry. However, insufficient evidence was provided to demonstrate the recommended dietary incorporation levels of 5000 to 10000 mg/kg would reduce the pH sufficiently to produce a noticeable decrease in ammonia production.

8.2. Addition of benzoic acid to feed at concentrations greater than the maximum recommended level did not affect meat quality and caused only minor changes to sensory properties of the meat.

8.3. The product VevoVitall® is well tolerated by pigs when added to diet at concentrations within the range 5000 - 10000 mg/kg.

8.4. Microbiological examination showed that supplementation of diet with benzoic acid at concentrations greater than the recommended range reduced numbers of some bacteria in the faeces of pigs. Although the shedding of *Salmonella* spp. was not directly addressed, the trend to reduced numbers of enterobacteriaceae would be very unlikely to create conditions favouring the growth and excretion of added numbers of human enteropathogens.

8.5. For user safety, a NOEL was not identified for inhalation toxicity, but a non-dusting formulation could effectively eliminate operator exposure by inhalation. VevoVitall appears to be irritant, and should be appropriately labelled.

8.6. With regard to consumer safety, human exposure to benzoic acid and its metabolites from animal feed use of benzoic acid is likely to be only a small part of the total exposure from all sources, including endogenous production and direct addition of benzoic acid to foods. The SCAN estimated this contribution to 0.25% of the ADI and hence concludes that there is no risk for the consumer.

8.7. The proposed use of benzoic acid on animal feed is unlikely to have adverse effects on the environment directly attributable to the product.

9. GENERAL COMMENT

SCAN notes that direct acidification of slurry is also possible. There is no evidence to show any advantages of use of benzoic acid as a urine acidifier through animal feed in comparison to the direct acidification of the slurry.

SCAN has a more general concern that acidifiers like benzoic acid, designed to address an immediate problem of ammonia production, only mask and do not address the underlying problem of incomplete nitrogen capture and the consequent pollution of soil and groundwater.

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