EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Scientific Opinions
**C3 - Management of scientific committees II; scientific co-operation and networks**

# Report of the Scientific Committee on Animal Nutrition on the use of potassium diformate (Formi$^{TM}$LHS) as feed additive

## (adopted on 22 March 2001)

## 1. TERMS OF REFERENCE

The Scientific Committee for Animal Nutrition is requested to give an opinion to the following questions:

1.1. Does the use of potassium diformate [$KH(COOH)_2$] under the conditions proposed in the feedingstuff for piglets and pigs for fattening improve animal production, in particular by affecting the gastro-intestinal flora?

1.2. Is the use safe to piglets and pigs for fattening?

1.3. Can it result in development of resistance in bacteria to prophylactic or therapeuthic drugs

1.4. Do the toxicology studies allow to conclude that the proposed use does not present risks

- For the consumer
- For the user/worker

1.5. What is the metabolic fate of potassium diformate in pigs? Does this use result in the presence of residues in animal tissues? If so, what is the qualitative and quantitative composition of these residues? Could these residues be harmful to the consumer?

1.6. What are the nature and the persistence of the excreted products derived from potassium diformate? Can these products be prejudical to the environment?

## 2. THE DATA PROVIDED BY THE COMPANY

The company has provided the working group with the following dossiers:

- Dossier for Formi LHS (Enclosure 1 to 3)
- Supplementary Dossier for Formi$^{TM}$LHS
- Supplementary Dossier for Formi$^{TM}$LHS; Enclosure 1 and 2, May 1998

- Supplementary Dossier for Formi$^{TM}$LHS; Enclosure 2, Volumes 1 to 3
- Supplementary Dossier of May 1999, Encl. 1 and 2
- Supplementary Dossier 4 of Nov. 1999, Conclusions
- Dossier for Formi$^{TM}$LHS, Summary on Section IV, Dec. 1999
- Supplementary Dossier of February 2000 for Formi$^{TM}$LHS
- Supplementary Dossier no 6, April 2000
- Supplementary Dossier no 7, May 2000
- Supplementary Dossier no 8, September 2000
- Supplementary Dossier no 9, October 2000
- Supplementary Dossier no 10, November 2000
- Recommended Doses for Inclusion of Potassium Diformate in Swine Diets (a separate document, November 2000)
- Supplementary Dossier 10, January 2001
- Clarification of recommended levels of use in reply to SCAN comments of 08 February 2001

## 3. DESCRIPTION OF THE PRODUCT

Potassium diformate (KCOOH*HCOOH) is a mixture of potassium formate and formic acid. The molecules are linked by a hydrogen bond between the hydroxyl group of the potassium formate molecule and the carbonyl group of the formate. The commercial product Formi$^{TM}$LHS contains 98% potassium diformate, 1.5 % silicate and 0.5% water: Physically the product is a coarse crystalline free flowing preparation with a particle size of no less than 70 micron.

The product is used as a growth promoter. The proposed inclusion rate in feed is:

- 0.6% to 1.8% for piglets until 2 months of age
- 0.6% to 1.2 % for growing/finishing pigs until slaughter

## 4. EFFICACY TRIALS

Several studies to measure efficacy of Formi$^{TM}$LHS for piglets and pigs for fattening were carried out. (see Table 1 hereafter). Four of them were done as dosage/response studies, one was the tolerance study.

Feed intake, daily gain and feed/gain ration were monitored in these studies. The highest effects of potassium diformate on daily weight gain were measured with piglets. The higher weight gain of pigs resulted primarily from increased feed intake and improved digestibility of some nutrients (e.g. crude protein). Because of the

higher feed intake, feed/gain ration was improved to a smaller extent in comparison with daily gain (table 1).

For piglets up to two months of age, significant results were observed at different levels of inclusion. However, the addition of Formi$^{TM}$LHS showed three significant results only at levels of 1.8% to 2.0% of diets, with increased daily weight gain of the animals and improved feed/gain ratio, respectively.

**Table 1:** Summary of experimental studies concerning the efficacy of Formi$^{TM}$LHS in piglets and pigs (Control=100%, changes compared to control group) (* $p<0.05$)

| Experiment no. | Experimental question | | Weight Range (kg) | No. of pigs per group | Level of Formi$^{TM}$LHS (%) | Feed intake (%) | Effect of Formi$^{TM}$LHS Daily gain (%) | Feed/ Gain (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | Piglets, Dosage/response Study | | 6 to 29 | 24 | 0.65 | +7 | +10 | -3 |
| | | | | 24 | 1.30 | +6 | +11 | -5 |
| | | | | 24 | 1.95 | +10 | +16* | -6* |
| 2 | Piglets, Dosage/response Study | | 7 to 28 | 6 | 0.4 | +6 | +7 | -1 |
| | | | | 6 | 0.8 | +4 | +5 | -3 |
| | | | | 6 | 1.2 | +10 | +11 | -1 |
| | | | | 6 | 1.6 | +12 | +16 | -4 |
| | | | | 6 | 2.0 | +15* | +22* | -6* |
| | | | | 6 | 2.4 | +5 | +12 | -7* |
| | | | | 6 | 2.8 | +10 | +15 | -6* |
| 3 | Piglets, Dosage/response Study | | 9 to 21 | 20 | 0.6 | +7 | +25* | -9* |
| | | | | 20 | 1.2 | +8 | +30* | -13* |
| | | | | 19 | 1.8 | +15* | +41* | -13* |
| 4 | Slaughter pigs, Dosage/response Study | | 7 to 105 | 12 | 0.65 | +1 | +5 | -4* |
| | | | | 12 | 1.30 | +1 | +3 | -3 |
| | | | | 12 | 1.95 | 0 | +6 | -5* |
| 5 | Piglets, Energy low (13.0 MJME/kg) | | 7 to 28 | 12 | 1.8 | +1 | +8 | -6 |
| | | | | 18 | 1.8 | +8 | +18* | -6 |
| | Piglets, Energy high (14.0 MJME/kg) | | | 12 | 1.8 | -1 | -1 | -3 |
| | | | | 18 | 1.8 | -1 | +1 | +1 |
| 6 | Challenge trial, piglets | | 6 to 10 | 5 | 1.8 | -4 | +3 | +9 |
| 7 | Piglets, compared with growth promoters (avilamycin/tylosin) [1)] to 2nd week ,[2)] 3rd-7th week | | 7 to 30 | 160 | 1.8[1)]/1.2[2)] | -2 | +5 | -4* |
| | | | | 160 "Low protein" | 1.8[1)]/1.2[2)] | -2 | +4 | -3 |
| 8 | Slaughter pigs, Effects in respect to time | | 6 to 26 | 24 | 1.6 | +3 | +7 | -4 |
| | | | (6 to 26) 26-95 | 12 | (0) 0.8 | +2 | +6 | -3 |
| | | | | 12 | (1.6)/0 | -1 | -1 | +3 |
| | | | | 12 | (1.6) 0.8 | +2 | +3 | 0 |
| | | | 6 to 95 | 12 | 0/0.8 | +2 | +4 | -3 |
| | | | | 12 | 1.6/0 | +2 | +1 | +1 |
| | | | | 12 | 1.6/0.8 | +2 | +4 | -1 |
| 9 | Tolerance study, 30 days | | 22 to 45 | 6 | 1.2 | +4 | +7 | -3 |
| | | | | 6 | 2.4 | +6 | +14 | -8* |
| | | | | 6 | 6.0 | -5 | +5 | -10* |
| 10 | Slaughter pigs | | 23 to 60 | 24 | 0.6 | +2 | +6 | -4 |
| | | | 60 to 105 | 24 | 0.6 | +2 | +1 | +1 |
| | | | 23 to 105 | 24 | 0.6 | +2 | +3 | -1 |
| 11 | Slaughter pigs, 5 experiments in commercial farms | 1 | 21 to 60 | 8 | 0.8 | -8 | 0 | -8 |
| | | | 21 to 106 | 8 | 0.8 | -1 | +4 | 0 |
| | | 2 | 23 to 60 | 8 | 0.8 | 0 | +9 | -9 |
| | | | 23 to 104 | 8 | 0.8 | -1 | +3 | -5 |
| | | 3 | 24 to 60 | 9 | 0.8 | -1 | +1 | -2 |
| | | | 24 to 102 | 9 | 0.8 | -1 | -3 | +1 |
| | | 4 | 24 to 60 | 9 | 0.8 | 0 | +1 | -1 |
| | | | 24 to 104 | 9 | 0.8 | 0 | +1 | -1 |
| | | 5 | 21 to 60 | 9 | 0.8 | -1 | +3 | -4 |
| | | | 21 to 107 | 9 | 0.8 | +2 | +3 | -2 |
| 12 | Slaughter pigs | | 23 to 97 | 12 | 1.5 | 0 | +3 | -3* |
| 13 | Slaughter pigs | | 27 to 104 | 32 | 0.6 | +2 | +3 | -1 |
| | | | | 32 | 1.2 | +3 | +6* | -3 |
| 14 | Slaughter pigs | | 23 to 105 | 12 | 1.8 | +2 | +3 | -1 |

Limited numerical increases in daily weight gain were observed during fattening period of pigs, but only in one case did this reach significance.

In conclusion, efficacy has been demonstrated in piglets with doses ranging from 1.8% to 2.0%. Regarding the lower dosages proposed by the Company for piglets (0.6%), and the doses recommended for growing/finishing pigs (0.6% to 1.2%), although there was a tendency for improved growth response, the efficacy was not demonstrated.

## 5. EFFECTS ON THE INTESTINAL MICROFLORA

The following studies have been performed on the effects of Formi$^{TM}$LHS on the gastrointestinal microflora of pigs and piglets:

(1) A feeding study using a 1.2 % Formi$^{TM}$LHS concentration with or without copper (150 ppm) and a standard feed without either supplement as a control. Each treatment group consisted of six animals. Different sections of the intestinal tract of piglets (slaughtered 4 weeks after weaning) were analysed for ATP concentration as an indicator of microbial activity. According to the results the amount of ATP in the stomach was significantly lower in Formi$^{TM}$LHS-treated pigs (0.82 μg $g^{-1}$ with Formi$^{TM}$LHS alone, 0.90 μg $g^{-1}$ with Formi$^{TM}$LHS combined with copper) than in the controls (2.09 μg $g^{-1}$). When Formi$^{TM}$LHS was combined with copper there was a decrease in ATP also in the small intestine (7.42 μg $g^{-1}$in the ileum of controls, 5.63 μg $g^{-1}$in the treated group). In the caecum there was an increase in the ATP from the control level of 13.1 μg $g^{-1}$ to 17.8 and 20.6 μg $g^{-1}$in the groups treated with Formi$^{TM}$LHS, either alone or combined with copper, respectively. Although there were large animal to animal variations there was a tendency of lactobacilli to increase in the small intestine and of coliforms to decrease throughout the intestinal tract, as a result of Formi$^{TM}$LHS-feeding (without copper), while the total amount of lactic acid bacteria seemed to decrease as a result of Formi$^{TM}$LHS and copper treatment. The observed differences were in the order of one log cycle the actual bacterial numbers in the control animals ranging between log 7.5 and log 9.5 for lactobacilli, and between log 4 and log 7 for coliforms, depending on the intestinal location).

(2) In another study the effects of 1.8% Formi$^{TM}$LHS on the faecal microbial profile of piglets were analysed. The feed used was a standard Copper containing ("160 units") starter piglet diet. The controls received this feed without the Formi$^{TM}$LHS supplementation. The trial was performed three times using each time 12 piglets in the control and test groups. The trial started at the weaning of piglets at 4 weeks' age, and continued for further 29 days. Faecal samples were analysed regularly throughout the study. In addition, on day 7 half of the piglets were killed and different segments of the gastrointestinal tract were analysed bacteriologically. On day 29, the same procedure was applied to the rest of the animals. The main finding was that there were significantly lower numbers of lactic acid bacteria and lactobacilli in the faeces (≥ $10^9$ cfu $g^{-1}$in controls, appr. $10^8$ cfu $g^{-1}$in the test group). While the levels of enterococci were not affected, there was a

decrease in the numbers of Enterobacteriaceae towards the end of the experiment (a gradual one log cycle reduction from the level of $10^7$ cfu $g^{-1}$ in controls between days 7 and 21). Also there was a tendency for total anaerobes to decrease (less than one log) in comparison to controls. The bacterial numbers in the digesta from different intestinal sections obtained from the autopsies reflected the situation observed in faeces.

(3) An experiment was performed with a concentration of 1.2% of Formi$^{TM}$LHS in the diet and with piglets of 24.1 kg of average weight at the start of the study. The control and test group each consisted of eight animals. The growing period was up to 7 weeks, while finishing period continued till the animals reached the average 104 kg live weight (controls 3 months, treated group 2.5 months). Microbial analyses were performed both during the experimental period and from the intestinal samples obtained during the slaughter. Only coliforms were analysed, and a significant overall reduction in their numbers were observed in duodenum and jejunum (from levels of appr. $10^4$ cfu $g^{-1}$ to 5 x $10^2$ cfu $g^{-1}$). In rectum the effect was not as prominent (less than one log cycle from the level of $10^5$ cfu $g^{-1}$ in the controls)

(4) In a study with three concentrations of Formi$^{TM}$LHS (0.9%, 1.8% and 2.7%), the intestinal contents were analysed after slaughter (40-41 days of age). The control and test groups each consisted of six animals. No further details regarding the duration of treatment, sex, race or rearing of the pigs are given in the report. According to the results, a decrease of coliforms (ileum and rectum), lactobacilli (ileum and duodenum) and a reduction of total aerobes and especially of total anaerobes in ileum and rectum were observed. The effects were most prominent (one log or more) with the highest dose. Small numerical decreases were observed with lower doses, but a clear dose-response relationship was missing.

(5) An experiment[1] with weaned piglets (21-24 days old), fed for 6 - 10 days with 1.8% Formi$^{TM}$LHS was performed. The test group consisted of 12 and the control group of 11 piglets. Data obtained from 6 animals slaughtered before the start of the feeding were used as a baseline, which both the test group and controls were compared to. A statistically significant decrease in the numbers of total anaerobes, aerobes, lactobacilli and especially of *E. coli* (log 8.07 before the start of the experiment, log 6.12 in Formi$^{TM}$LHS-treated animals compared to log 7.35 in the controls) was observed in the small intestine.

(6) A challenge trial has also been performed with pathogenic *E. coli* strains. The concentration of Formi$^{TM}$LHS was 1.8% in the basal diet. After 7 days the piglets were each dosed with $10^9$ of *E. coli* O149, K89 and K88ac. Only one of the piglets in the Formi$^{TM}$LHS had diarrhoea of a short duration, and none excreted pathogenic *E. coli* in a marked contrast to the control group with three cases of scouring and all but one (out of five) shedding the administered strain.

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[1] Complete results of the study presented in Heberler *et al.*, 2000. Proceedings of the Society of Nutrition Physiology. **9:** 63

In conclusion, despite the variations between the individual experiments, Formi ™ LHS in concentration range of 1.2% to 2.0% has a tendency to decrease the bacterial counts, especially *Enterobacteriaceae*, and lactobacilli. No data of the effects of the 0.6% inclusion level have been provided. Although the observed changes in the intestinal flora are consistent with a reduced immunological challenge to the host digestive tract, it is not possible to conclude that they are causally related to the observed improvements in animal performance. There was no indication of Formi™LHS affecting the general balance of intestinal microflora or reducing/increasing any group of bacteria to drastically different levels from those normally observed, and thus the observed effects are not a safety concern.

## 6. SAFETY TO TARGET ANIMALS

### 6.1. Tolerance test

The tolerance study was done on growing/finishing pigs at the maximum inclusion level of 6% for 90 days. Palatability problems prevented higher concentrations being employed.

A total of 24 crossbreed [(Norwegian Landrace x Yorkshire) x Norwegian Landrace] growing-finishing pigs from five litters with an average initial weight of 22.4 kg were used. Pigs were allotted by litter, initial weight and sex to four dietary treatments. There were four pigs from four litters and eight pigs from one litter.

The treatments consisted of a conventional control diet containing no Formi™LHS and three test diets containing 1.2%, 2.4% or 6% Formi™LHS (these concentrations correspond to approximately 500, 1000 and 2500 mg/kg bw/day). The energy value of the feed was calculated according to the Danish NE system. Water consumption per pen was recorded weekly from day 53 of the experiment and through the study.

Blood samples were taken at days 0, 28, 58 and 88. Samples were analysed for content of haemoglobin and leucocytes. The serum was analysed for glucose, protein, urea, creatinine, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin, sodium, chloride, potassium and calcium. Heparinised blood sample was analysed for pH, partial pressure of carbon dioxide ($pCO_2$), oxygen-saturation ($pO_2$), bicarbonate ($HCO_3$) and standard base excess.

At day 25 of the experiment, faecal samples were taken for formate analysis from pigs on the control and the 6% Formi™LHS. The method used for analysing formic acid is based on a kit from Boehringer Mannheim (Cat 979 732) and is based on a spectrophotometric method where formate is oxidised to bicarbonate with formate dehydrogenase and $NAD^+$. The amount of NADH is then measured. A digestibility study based on grab sampling was conducted using 8 crossbreed growing-finishing pigs from two litters. The analysis was performed in duplications.

Two pigs from each treatment group were autopsied on days 30, 60 and 90 after the experiment initiated. Samples of kidney, liver and muscle were

taken for formate analysis. Also samples of heart muscle (day 60) samples of heart and lung (day 90) were also taken for formate analysis. Stomach and gut were examined for lesions.

Statistical analyses were performed on the data collected from day 0 to day 60 using the general linear model procedure (GLM) of SAS (1990) for a complete randomised block design.

The results can be summarised as follows:

Blood chemistry and haematology. Increasing dietary Formi$^{TM}$LHS levels up to 6% (5 times the recommended dosage), did not influence the acid base balance of pigs after receiving the diets for 28 days. At day 58, pigs receiving 6% Formi$^{TM}$LHS in the diet showed slightly higher $pCO_2$ values in blood and consequently slightly lower serum pH values that the other pigs.

Blood parameters at day 28 of the experiment showed increased serum K and Na levels, at the 6% Formi$^{TM}$LHS dose. At day 58 and day 88 the K and Na levels were normal for all treatments.

At day 28 the 2.4% and 6% Formi$^{TM}$LHS diet gave a significantly lower serum urea level than the control and 1.2%, and there was a dose-dependent increase in serum creatinine in the treated groups. By day 58 the urea levels were slightly elevated in 1.2% and 2.4% treatment groups, while no differences were observed between the 6 % group and controls, or in the serum creatinine between any of the groups.

Adding up to 6% Formi$^{TM}$LHS in the diets for 88 days had no effect on leucocyte levels in blood.

Pathology. At the dose of 6%, Formi$^{TM}$LHS increased the relative kidney weight at day 30 and day 90. With the 2.4 % dose, the relative kidney weight increased at day 60 but this effect was not seen at day 90.

At the 6% dose level (10 times the lowest dose recommended by the Company), preliminary or moderate hyperkeratosis was observed in the oesophagal part of the stomach at 60 and 90 days. Similar but milder effects were observed also in controls. (see 6.3)

## 6.2. Effect of Formi$^{TM}$LHS on the water intake

According to the theoretical estimates based on the literature data Formi$^{TM}$LHS could increase the daily water consumption in grower pigs between 2.6% and 8% when administered at the level of 1.2%. The figures are mainly based on estimates given in the literature[2], and specifically on a

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[2] Brooks, P.H. , Carpenter, J.L., Barber, J. & Gill, B.P. (1989) Production and welfare problems relating to the supply of water to growing-finishing pigs. Pig Veterinary Journal 23, 51-56.

MroZ, Z., Jongbloed, A. W., Lenis, N.P. & Vreman, K. (1995) Water in pig nutrition: Physiology, allowances and environmental implications. Nutrition Research Reviews, 137-164

study, in which the effects of potassium (in the form of $KHCO_3$) on the water consumption of piglets was specifically evaluated starting at the age of 35 days and continuing for another 35 days. According to the results the piglets tolerated well the increase of potassium up to 19 g /kg feed (the highest dose tested). An average daily increase of 35 ml /each extra g of potassium was observed in the water consumption. No treatment-dependent effects on the serum concentrations of vasopressin, rennin, sodium, potassium or protein were observed.

In contrast to these figures, Formi$^{TM}$LHS (at the tested dosages of 1.2, 2.4 and 6 %), produced during the days 53-60 of the tolerance test (first time of measurement) a considerable increase in water consumption (7.2, 8.8, and 10.6 liters per pig per day, respectively) compared to controls (5.6 litres per pig per day). Between 60 and 90 days, water intake in treated pigs at each dose level was doubled in comparison to controls (5.0 vs. 10.9 per pig per day). However, this study was not designed to specifically monitor the water consumption, and the water spillage was not controlled or eliminated from the estimates.

Subsequently two studies designed specifically to follow the water consumption in different climatic conditions, were performed. In a Norwegian study 24 crossbreed weaning pigs from 5 litters were used in a 14 day experiment. The average temperature was 19.6 °C. The water for each pen (housing four pigs) came from a 20 l tank filled daily, and the water usage was estimated by weighing the left over water in the tank. The results are listed in table 3.

Table 3. Effect of 1.2 % Formi$^{TM}$LHS in diets on water usage on young pigs (Norwegian study).

| | **Formi$^{TM}$LHS in diets** | | **SEM** | **P-value** |
|---|---|---|---|---|
| | 0 | 1.2% | | |
| No. of pigs | 12 | 12 | - | - |
| Initial weight (kg) | 15.3 | 15.3 | 0.4 | NS |
| Final weight (kg) | 25.9 | 26.0 | 0.7 | NS |
| Average daily feed intake (kg) | 1.05 | 1.15 | 0.01 | 0.5 |
| Total feed intake (kg/pig) | 14.74 | 16.08 | 0.13 | 0.05 |
| Average daily water intake, L/pig | 2.31 | 2.42 | 0.06 | NS |
| Total water intake, L/pig | 32.2 | 32,2 | 0.9 | NS |
| Water intake/feed intake, kg/kg | 2.19 | 2.12 | 0.04 | NS |

Another study with a higher dose (1.8%) of Formi$^{TM}$LHS was conducted in Spain. The temperature range during the experiment was 30 - 32 °C. The number of piglets was initially 20, the average initial weight was 8 kg, and the duration of the trial was three weeks. During the first three days of the trial two piglets (one of the controls and one from the treated group) died of

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Joergensen, L. & Rasmussen, E.S. (1994) Kalium i foder til smågrise. Info svin, svinefaglig database, landsudvalget for svin, Danske slagterier. No 275, pp 5

diarrhoea. The pigs were housed in metabolic cages (two pigs per cage). The arrangements for measuring the water consumption were similar to those in the Norwegian study. In addition also the amount of spilled and leaked water was measured. The results are listed in Table 4. In addition to animal performance and water consumption, also slurry output and dry matter, as well as relative kidney weights after the trial, were studied

Table 4. The effect of Formi$^{TM}$LHS on the water intake of piglets (Spanish study). The values represent the whole experimental period, except for the 7 - 20 day water intake/feed intake ratio.

| | **Formi$^{TM}$ LHS in diets** | | **SE** | **P-value** |
|---|---|---|---|---|
| | 0 | 1.8% | | |
| No. of pigs | 9 | 9 | - | - |
| Initial weight (kg) | 7.62 | 7.63 | 0.06 | 0.97 |
| Final weight (kg) | 14.54 | 15.89 | 0.53 | 0.15 |
| Average daily gain (g) | 346 | 413 | 27.9 | 0.16 |
| Average daily feed intake (g) | 429 | 496 | 31.7 | 0.21 |
| Average daily water intake, g /pig* | 1273 | 1430 | 109.2 | 0.38 |
| Water intake/feed intake kg/kg (7 - 20 days) | 3.10 | 3.09 | 0.17 | 0.97 |
| Water intake/feed intake, kg/kg (0 - 20 days)* | 2.84 | 3.01 | 0.16 | 0.51 |

*In the 0-20 day water intake and water/feed intake ratio calculations the data from two cages, where one of the piglets had died, were treated as missing values

In the control group the average daily slurry production was 782 g per pig, and in the Formi$^{TM}$LHS-treated group 851 g. The corresponding figures for dry matter in the slurry were 10.45% and 15.21%.

The relative kidney weight in the control group was 0.28% (right kidney) and 0.26% (left kidney), while the indexes in the Formi$^{TM}$LHS-treated group were 0.28% and 0.29%. These differences were not statistically significant.

### 6.3. Studies on the hyperkeratinisation of the stomach epithelium

Hyperkeratinisation of the stomach epithelium was the main pathological finding in the pig tolerance study. These stomach alterations may be caused by small feed particle size, low pH in stomach contents, dietary ingredients and other factors. The grading of the alterations varies between 0 (unaffected white and smooth mucous membrane) and 10 (very severe cardia stenosis etc.). Scores between 0 and 3 are considered irrelevant, as such lesions may occur under normal conditions.

In addition to the tolerance study, eight other experiments were done to specifically assess the effect of Formi$^{TM}$LHS on stomach alterations in piglets and pigs (Table 5). In some experiments (e.g. exp. 1, 4, 6, 7 and 8, Table 5) there is an impression of a higher score of stomach alterations of Formi$^{TM}$LHS added animals. However, even in these experiments the highest scores did not differ from those seen also in the controls. In other experiments (2, 3 and 5, Table 5) there are lower scores after supplementation.

SCAN is of the opinion that score values would be better characterised by the median than by the arithmetic mean. However, it can be concluded that the incidence and severity of stomach lesions in the Formi$^{TM}$LHS-treated groups did not differ significantly from those seen in controls.

**Table 5:** Score of stomach observations (0-10) in trials with Formi$^{TM}$LHS in piglets and pigs.

| Experiment No. | Pig Category | Day of examination | Number of animals per treatment | Formi$^{TM}$LHS dosage (%) | Average score | Highest score |
|---|---|---|---|---|---|---|
| 1 | Piglets | 28 | 6<br>6 | 0<br>1.2 | 2.3<br>3.8 | 6<br>6 |
| 2 | Piglets | 7<br>29 | 9<br>9<br>9<br>9 | 0<br>1.8<br>0<br>1.8 | 2.2<br>1.3<br>2.3<br>2.1 | 4<br>5<br>5<br>5 |
| 3 | Pigs | 85 | 32<br>32<br>32 | 0<br>0.6<br>1.2 | 1.8<br>1.7<br>1.5 | 3<br>4<br>3 |
| 4 | Piglets | 28 | 8<br>8 | 0<br>1.8 | 2.1<br>2.8 | 3<br>5 |
| 5 | Piglets | 12 | 4<br>4<br>4 | 0<br>0.9<br>1.8 | 1.8<br>0.8<br>0.3 | 3<br>2<br>1 |
| 6 | Piglets | 14 | 25<br>25 | 0<br>1.2 | 1.1<br>1.8 | 3<br>3 |
| 7 | Piglets | 28 | 8<br>8 | 0<br>1.2 | 1.1<br>2.4 | No information |
| 8 | Pigs | 77 | 12<br>11<br>11<br>12 | 0<br>1.8<br>3.6<br>5.4 | 0.9<br>0.8<br>1.4<br>1.7 | 4<br>1<br>4<br>5 |

## 6.4. Conclusions

In the tolerance test on growing/finishing pigs even the highest dosage of Formi$^{TM}$LHS applied (6%) produced only slight or transient differences in different clinical parameters, such as increased serum potassium and sodium levels at day 28 of the experiment. Regarding the post mortem findings, an increased relative kidney weight was observed at dose levels of 6% and 2.4%. With the latter dose, the effect was not observed at day 90.

No specific tolerance study in piglets was performed. A dose response study on piglets with doses ranging from 0.4% to 2.8% (Table 1, experiment N°2) has been reported and shows an improved animal performance. Although this study is not sufficient to establish an adequate safety margin for the piglets fed the highest level of use of Formi$^{TM}$LHS recommended by the Company (1.8%), it allows the lowest level recommended for piglets (0.6%), to be considered as safe.

Unexplained increased water intake occurred during the tolerance test in pgs for fattening. In two subsequent trials, no increased water intake could be observed in piglets fed with 1.2% or 1.8% Formi$^{TM}$LHS supplementation

and in both North and South Europe climatic conditions. The possibility of increased water intake as a result of prolonged feeding of growing/finishing pigs fed with the highest dose recommended by the Company (1.2%) has not been ruled out. The Company recommends reduction of the Na content of the feed to a maximum of 0.15%.

The results of the tests performed specifically to assess the possible induction of changes to stomach mucosa by Formi$^{TM}$LHS, do not suggest that Formi$^{TM}$LHS, at the dose levels up to 1.8% would significantly increase the incidence or severity of hyperkeratosis in piglets or pigs for fattening.

## 7. INDUCTION OF RESISTANCE IN BACTERIA TO PROPHYLACTIC OR THERAPEUTIC DRUGS

Formic acid, as other short chain organic acids, has antimicrobial properties and is used in antimicrobial preparations. Bacteria can adapt physiologically to increased concentrations of organic acids, but this phenomenon is transient and not a permanent adaptation.

None of the actual known antibiotic acts through formation of formic acid or formate. The risk of Formi$^{TM}$LHS interfering with veterinary or human therapeutic measures is remote.

## 8. POTENTIAL RISKS OF POTASSIUM DIFORMATE AND ITS METABOLITES

### 8.1. Toxicological tests

#### *8.1.1. Genotoxicity tests*

Bacterial reverse mutation tests, mammalian cell point mutation tests and tests for chromosome aberration have been performed on Formi$^{TM}$LHS. Concentrations of up to 5000µg of Formi$^{TM}$LHS per plate did not induce reverse mutations in *Salmonella typhimurium* strains TA100, TA98, TA1535 and TA1537 and in *E. coli* strain WP2 *uvrA* in the presence and the absence of a metabolic activation system. In a mammalian cell point mutation test no induction of mutations at the *tk* locus of mouse lymphoma L5178Y cells was observed at doses up to 1.302 µg per ml (10 mM, the highest tested level in a cytotoxicity range finding study), in the presence or absence of a metabolic activation system.

No chromosomal aberrations were observed in cultured human peripheral blood lymphocytes with doses of Formi$^{TM}$LHS up to 10 mM (again regardless of metabolic activation). In the absence of the metabolic activation system the two highest dose resulted in a 55% reduction of mitotic index indicating that a toxic concentration was approached. Neither was there any induction of micronuclei in the bone marrow polychromatic erythrocytes of rats intravenously dosed with 12.5, 20 and 50 mg of Formi$^{TM}$LHS per kg per day. Also here the highest dose induced some clinical signs (lethargy, prostration,

abnormal gait and breathing) making higher dosing impossible. At this level also the ratio of polychromatic erythrocytes to normochromatic ones was reduced indicating toxicity to bone marrow cells.

*8.1.2. Acute oral toxicity*

The acute oral toxicity studies on rat an mouse have been performed. In rats the $LD_{50}$ of Formi ™ LHS was concluded to be more than 2000 mg per kg body weight. No deaths of male mice occurred at a dose level of 2 000 mg per kg, while 2 females out of the group of 5 died. Consequently an actual $LD_{50}$ trial was performed with female mice, and the resulting value was 2988 mg per kg body weight. The main autopsy findings in the mice that died during the study were bilateral distension of the uterus, gaseous distension in the gastrointestinal tract (one mouse, dose 2000 mg per kg), and darkening of the gastric mucosa and discoloration of liver and small intestine (two mice dosed at 2700 mg /kg).

*8.1.3. Subchronic and chronic oral toxicity*

A range finding 13 week oral toxicity study in mouse has been performed with Formi™LHS mixed in the diet in a calculated dose range of 600, 1200 and 3000 mg per kg per day. Even the highest dose was well-tolerated and no adverse clinical effects or histopathological findings were observed.

Combined chronic toxicity and carcinogenicity studies performed in both rats (104 week study) and mice (80 week study) with Formi™LHS doses of 50, 400 and 2000 mg/kg/day).

No treatment related incidence of tumours was observed in either of the studies In rats, increased incidences of basal/squamous cell hyperplasia of the stomach were observed in the two top dose groups. In mice, low grade limiting ridge hyperplasia in the stomach of some high dose males. Accordingly, regarding the changes in the stomach epithelium, the NOAEL values for rats could be defined as 50mg/kg/bw/day for rat and 400mg/kg/bw/day for mouse. No treatment related macroscopic or histopathological findings were recorded in either animals.

*8.1.4. Skin and eye irritation studies, skin sensitisation, inhalation toxicity*

Rabbit skin and eye irritation tests and a Guinea pig skin sensitisation assay (Magnusson-Kligman test) have been performed. While no indication of serious skin irritation was observed, the ocular irritation was sufficient for the risk phase R41 "Risk for serious damage to the eye". No signs of delayed dermal hypersensitivity were detected in the Guinea pig skin sensitisation test.

In an acute 4h rat inhalation toxicity study performed no deaths occurred during the exposure despite observed reduction in breathing frequency. The LC50 was concluded to be more than 5.16 g per $m^3$

In conclusion, there was no evidence of genotoxicity of Formi™LHS. The acute oral toxicity was low, and no adverse chronic effects at the tested dose levels were detected. No indications of skin irritation or delayed type of

hypersensitivity were observed. The only findings were the eye irritation in the rabbit test and the thickening of the stomach epithelium in the long term rat study. The results of mouse and rat carcinogenicity studies have been negative.

## 8.2. The risks to worker or handler of the product

The dusting properties of Formi$^{TM}$LHS are reported to be low (10.2 g dust released per kg during "rough handling of the product") with the particle size fraction < 70 µm being less than 1 %.

From the toxicological data it is apparent that the main risk for the workers is the eye irritation caused by Formi$^{TM}$LHS. The product is labelled with the risk phrase R41 (risk of serious damage to the eye). Also the liberation of gaseous formic acid may cause irritation of the respiratory system. In the safety data sheet protective measures (eye protection, gloves, mask with gas filter E) are recommended by the Company.

## 8.3. The risk to consumer

The consequence of the chemical characteristics of potassium diformate, namely the hydrogen bond between the hydroxyl group of the potassium formate molecule and the carbonyl group of the formate, is that the complex remains at the diformate form under acidic conditions and dissociates into formate and potassium ions under neutral or alkaline conditions.

Therefore, diformate, present as the salt under the acidic conditions of the stomach, is likely to dissociate in the neutral condition prevailing in the intestine and after absorption, in plasma and tissues. Formate is a normal endogenous metabolite.

A pharmacokinetic GLP-study of potassium diformate has been carried out after single oral dosing of pigs. Four females SPF pigs weighing 27-32.5 kg were dosed the test article at a rate of 6% in the feed with molasses added.

Totally, 11 blood samples were collected before dose (day –1) and 30, 60 and 90 min and 2, 3, 4, 5, 7, 12 and 24 h. Formate was analysed in plasma using a validated enzymatic analysis method. The lower limit of formate detection (LOD) was 10 µg/ml.

From the predose level of 1.9±1.9 µg/ml corresponding to the level of the endogenous formate, the plasma formate concentrations reached a $C_{max}$ of 385.4±80.7µg/ml at 4 h after dosing. Formate levels were below the LOD at 12 h after dosing in 3 of the 4 animals (the mean level 26.3±45.9µg/ml). After 24 h in all 4 animals the values were below the LOD ( the mean level 0.6±1.4 µg/ml). The $t_{½}$ for elimination was 2.73 h.

As a conclusion, formate is rapidly absorbed and eliminated. The percentage of absorption (i.e. bioavailability) has not been calculated in the studies described above.

The distribution of formate in various tissues as a result of Formi$^{TM}$LHS feeding was measured during the tolerance test using an enzymatic assay (see section 6.1). The effects of increasing dietary levels of Formi$^{TM}$LHS on the content of formate in loin muscle, liver and kidney are expressed in Table 6. Because only two animals were used for each time point and dose level, the results can be considered as indicative only.

Tissue levels of formate have also been measured in two other studies (sensory study on animal produce and a specific study on formate residues) on growing-finishing pigs (initial weights 24 -25 kg and the final weight 100 kg). In the sensory study the method applied was the enzymatic one used also in the tolerance test. A more sensitive chromatographic method[3] was applied in the actual residues studies. The dose applied was 0.7%. The results of this study are summarised in Table 7.

Table 6. Effect of increasing dietary levels of Formi$^{TM}$LHS on formate content in pig tissues (µg/g) and serum (µg/ml) (two animals per group)

| **Formi$^{TM}$LHS diets** | | | | |
|---|---|---|---|---|
| | **Control Mean** | **1.2 % mean** | **2.4% mean** | **6% mean** |
| **Formate in day 30 samples*** | | | | |
| No of animals | 2 | 2 | 2 | 2 |
| Loin muscle | < 20 | <20 | 21.3 | 151.3 |
| Liver | 42.2 | 49.1 | 45.9 | 113.6 |
| Kidney | 26.1 | 46.5 | 57.0 | 355.5 |
| **Formate in day 60 samples*** | | | | |
| No of animals | 2 | 2 | 2 | 2 |
| Serum | 106 | 99.5 | 153.5 | 305.5 |
| Loin muscle | 25.0 | 59.5 | 56.5 | 66.5 |
| Liver | 54.4 | 40.0 | 63.9 | 62.2 |
| Kidney | 48.8 | 49.6 | 81.4 | 111.4 |
| Heart | 23.8 | 39.3 | 54.7 | 72.6 |
| **Formate in day 90 samples*** | | | | |
| No of animals | 2 | 2 | 2 | 2 |
| Serum | 34.2 | 49.8 | 188.8 | 321.1 |
| Loin muscle | <20 | <20 | 22.8 | 37.4 |
| Liver | <20 | 25.2 | 21.2 | 27.3 |
| Kidney | 25.8 | 34.7 | 45.5 | 27.3 |
| Lung | 31.3 | 44.9 | 46.3 | 83.2 |
| Heart | 28.6 | 30.7 | 53.5 | 97.1 |

*The detection limit of the enzymatic assay method was 20 µg/g±1.0

[3] The accuracy of the chromatographic method has been evaluated with a recovery test using meat samples into which various amounts of formate had been added. According to the results the measurements are accurate in the concentrations exceeding 5 ppm.

Table 7. Formate levels in tissues of growing -finishing pigs fed with Formi$^{TM}$LHS

| Trial | Formi$^{TM}$LHS in the diet | Number of animals | Formate in tissues (µg/g) (mean ± standard deviation, when appropriate) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Muscle | Liver | Lung | Spleen | Kidney | Skin | Fat |
| Sensory evaluation study | 0 | 5 | < 20 | < 20 | - | - | < 20 | - | - |
| | 0.8 % | 5 | < 20 | < 20 | - | - | < 20 | - | - |
| Formate residue study (1) | 0 | 9 | < 5 | 18 ± 2 | 6 ± 2 | 7 ± 2 | 9 ± 2 | < 5 | < 5 |
| | 0.7 % | 9 | < 5 | 18 ± 2 | 5 ± 1 | 7 ± 3 | 12 ± 2(2) | < 5 | < 5 |

- = not tested

(1) The pigs were slaughtered about 24 - 26 hours after their last meal

(2) Statistically significant (P< 0.05) difference compared to control

The fact that two different methods that have not been intercalibrated have been used in the residue studies makes the direct comparison of results somewhat arbitrary.

The results from the tolerance study trial indicate an increase of formate levels in the tissues when the Formi$^{TM}$LHS level in the diet increases from 1.2% to 6.0%. The phenomenon is very prominent at the highest dosage but less apparent with the lower ones due to the limited number of animals tested. (two per feed concentration) and important individual variations. Moreover fat and skin have not been checked.

The results from the specific formate residue study are more consistent as 9 control and 9 treated animals were studied, and the main edible tissues sampled and tested for formate contents using a chromatographic method.

The daily intake of formate for a 60kg person resulting from the consumption of the standard reference diet (300g muscle, 100g liver, 50g kidney and 50g skin/fat) would be approximately 21mg when considering formate levels in control animals (endogenous formate), but 30mg using tested animals' tissues.

In contrast to these values, calculations based on the results from the formate residue study in Table 6 would give a daily dose of 4 mg per person. These figures are well within the ADI of formic acid (3 mg/kg bw/day) defined by JECFA[4].

[4] Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. WHO Food Additives Series, n°5, pp. 60-61, 1974, Geneva.

## 9. THE EXCRETED PRODUCTS, EFFECTS ON THE ENVIRONMENT

### 9.1. Faecal and urinary formate concentrations

The formate levels in faeces and urine were also measured in connection with the pig tolerance study. As expected, the formate content of the faeces was not affected by dietary Formi$^{TM}$LHS supplementation. Instead, in urine a dose dependent increase in the amount of formate was observed (300 µg and 2040 µg per g in the groups treated with 2.4 % and 6 % of Formi$^{TM}$LHS in the diet, respectively, compared to 150 µg per g in the control group). There was, however, no increase in the urinary formate at the dietary dose level of 1.2 %. Thus, regarding the proposed dose range (0.6 – 1.8 %), the additional environmental exposure to formate would be only marginal.

### 9.2. Ecotoxicological studies on potassium formate

A series of ecotoxicological studies with potassium formate on different aquatic life forms (fish, algae, insects, crustaceans etc.) have been performed. According to the results, the effects of potassium formate in the aquatic environment were only moderate, the 48h $LC_{50}$ for the most sensitive organism used, the copepod *Acartia tonsa*, being 531 mg/l. With the other test species the $EC_{50}$ or $LC_{50}$ were well above 1000 mg/l, or not definable.

In conclusion, provided that no other excreted metabolites exist, the extra urinary formate is not an environmental risk.

Regarding the effects of potassium, the reported increase in the urinary concentrations of potassium as a result of 1% Formi$^{TM}$LHS in the diet was from the level of 7.15 g/day (controls) to 8.27 g/day. In faeces, the corresponding figures were 4.85 g/day and 5.18 g/day. The calculated overall potassium increase in the excreta was 12%.

Although it is difficult to estimate the effects of Formi$^{TM}$LHS on the potassium levels in the pig excreta during the whole production period, due to variations in the recommended dosages and differences in the slurry output during the different production phases, it can be concluded, that the extra amounts of potassium would not drastically affect the environment, unless the pig population is very high.

## 10. GENERAL CONCLUSION

After reviewing the data provided by the company, SCAN concludes that potassium diformate, when used at the dose of 1.8% to 2.0%, improves the growth performance of piglets. It cannot be stated however, whether the effect is based on changes observed in the gastrointestinal microflora. Regarding the lower recommended dosage (0.6%) and the other target animal category, growing/finishing pigs, although there is a tendency for improved growth response, no sufficient statistically significant efficacy data have been presented.

Regarding the way of administration, it should be noted that if administered with wet feed, Formi$^{TM}$LHS is unstable, if water or sweet whey are used. If feed is administered in a water suspension or with non-acid whey with an almost neutral pH level, potassium diformate breaks down into formate and potassium ions. Under such conditions, pigs are no longer receiving the Formi$^{TM}$LHS as an additive, but rather a mixture of formic acid, formate and potassium.

Concerning safety of animals, on the basis of the data available, SCAN concludes that the product appears to be safe at the level of 0.6% for both animal categories. However, in order to fully demonstrate the safety of animals at the highest proposed level of the dose ranges, additional studies would be necessary. For piglets, a tolerance test based on the highest dose recommended by the company would be needed. For pigs for fattening, experimental data on the increased water consumption and its origin at the highest level of use (1.2%) recommended by the Company should be provided.

The use of the product does not result in the development of bacterial resistance against presently used prophylactic or therapeutic preparations.

According to the toxicological studies potassium diformate is of very low or moderate toxicity, and when handled according to the instructions given by the manufacturer, the compound as such should not present risks to either consumer or worker.

The only detectable residue of potassium diformate in animal products is formate, and the levels in tissues in finishing pigs fed at the lowest recommended dose level (0.6%) do not present a toxicological concern. There is however no sufficient information on the residue levels resulting from the higher dosages (1.2% for pigs for fattening, 1.8% for piglets), although the limited data available from the tolerance study suggest that the residues remain at a relatively low level.

Formate (and potassium) are also the only excreted products, and according to the ecotoxicological studies, the environmental effects of formate in the actual concentrations in excreta are negligible.