EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

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

Update of the opinion of the Scientific Committee for Animal Nutrition on the use of formaldehyde as a preserving agent for animal feedingstuffs of 11 June 1999

(Adopted on 16 October 2002)

1. BACKGROUND

The SCAN adopted an opinion on formaldehyde in June 1999 on the use of formaldehyde as a preserving agent for animal feedingstuff. This opinion updated a previous report of October 1995.

The Scientific Committee identified a number of shortcomings in the dossier presented and could not therefore conclude on the safety or the efficacy of the product when used in animal feedingstuffs.

In August 2000, the company submitted a supplementary dossier in reply to the SCAN opinion.

2. TERMS OF REFERENCE (FEBRUARY 2001)

The Scientific Committee for Animal Nutrition is therefore requested to consider the additional information provided by the company and to conclude on the efficacy and the safety of the product when used at the level recommended by the company.

3. THE PROPOSED USE AND CLAIMS ASSOCIATED WITH FORMALDEHYDE

In the resubmitted dossier the company has maintained the proposed dose (660 mg/kg feed) but limited the use of the additive to pigs, piglets and chickens for fattening, as specified in Table 1.

Table 1: Annex entry proposed by the company

EC No	Additive	Chemical formula and description	Species or category of animal	Maximum content (mg/kg of complete feed)	Other provisions
E240	Formaldehyde	CH_2O	Poultry, Pigs and piglets	660	For feeding stuffs, for reduction of pathogenic microorganisms; one administration

The company has not made any definite claim concerning the intended use of formaldehyde (sterilising agent, general preservative or a specific anti-Salmonella

additive?). However, it specifically states that no claim is made regarding prevention of recontamination by micro-organisms.

Consequently, SCAN understands that the intention is to use formaldehyde as a single decontamination treatment to reduce the numbers of harmful micro-organisms, particularly *Salmonella* spp., in feeds. The following opinion is based on that assumption.

4. INTRODUCTION

Formaldehyde has been previously assessed by SCAN (SCAN opinion, June 1999) as an antimicrobial feed additive specifically aimed at the elimination of *Salmonella* spp. in feeds. The additive was recommended by the company for all species and categories of animals at the dose level of 660 mg/kg. In 1999 SCAN concluded that the requirements of Council Directive 70/254/EEC were not fulfilled. The main reasons were the following:

(1) Although the company provided some evidence that formaldehyde at the proposed inclusion level is able to eradicate or substantially reduce the number of pathogenic bacteria such as *Salmonella* in animal feedingstuffs, the proposed dose did not protect against recontamination, nor did the proposed single dose take into account the considerable variations of feeds or feed ingredients.

(2) No data on target animal safety, except some data on broilers, were provided.

(3) Neither the user's safety nor the consumer safety had been adequately demonstrated

In July 2001 the company resubmitted their dossier providing answers to the points raised by SCAN. This opinion is based on the data in this new dossier and on the subsequent company response to the specific questions presented by SCAN during the re-evaluation process.

5. THE ANTIMICROBIAL EFFICACY OF FORMALDEHYDE

In the new dossier the company resubmits the original data showing the reduction of the numbers of *Salmonella* and other feed-associated bacteria as the result of formaldehyde treatment. Additional new data from two experiments are provided on the antibacterial effects of formaldehyde in artificially inoculated feed samples.

It should be noted that no studies have been reported demonstrating an actual reduction of occurrence of *Salmonella* in animals or animal products obtained from production facilities where formaldehyde has been used as feed additive.

5.1. The effects of formaldehyde treatment on Salmonella positive feeds

Among the material presented already in the original dossier of 1996 a series of *Salmonella* analyses from treated and untreated, originally *Salmonella*-positive feeds, was shown (Table 2). The feeds covered a wide range of different feedingstuffs with variable protein content.

Table 2. The effect of formaldehyde on *Salmonella* when applied at a level of 660 mg/kg under commercial conditions in a range of feedingstuffs

Feedingstuff, total quantity tested	Protein content (%)	*Salmonella* contamination in untreated samples		*Salmonella* contamination after formaldehyde treatment		
		Number of of-positive samples	*Salmonella* count (MPN/100g)	Number of positive samples	*Salmonella* count (MPN/100g)	Number of negative samples
Soybean meal, 1006 tonnes	44	27	7	0	<1	57
Soybean meal, 1224 tonnes	48	17	7	0	<1	85
Wheat bran, 1500 tonnes	16.0	Official samples positive	> 4	0	<1	6
Barley, 3800 tonnes	10.3	Official samples positive	> 4	0	<1	10
Wheat, 3000 tonnes	11.5	Official samples positive	> 4	0	<1	16
Palm kernel, 1000 tonnes	15.0	Official samples positive	> 4	0	<1	18
Palm kernel, 1000 tonnes	15.0	Official samples positive	> 4	0	< 1	12
Palm kernel, 1000 tonnes	15.0	3	>4	0	<1	12
Rape meal, 1000 tonnes	34.2	Official samples positive	>4	0	<1	18
Rape meal, 1100 tonnes	34.2	Official samples positive	> 4	0	<1	10
Rape meal 4000 tonnes	34.2	Official samples positive	>4	0	<1	15
Copra, 1040 tonnes	20.0	Official samples positive	>4	0	<1	8

MPN: most probable number

It can be concluded that the treatment had reduced the levels of *Salmonella* below the detection limit of the analytical method. The company also cites in the original dossier similar results obtained from commercial compound feed samples.

5.2. The antibacterial action of formaldehyde in artificially contaminated feeds

In the new dossier the company provides data on the effects of formaldehyde in feeds that had been artificially inoculated with either *Escherichia coli* or *Salmonella typhimurium*.

In the first experiment the feed was contaminated with *E. coli* ATCC 8739 at a calculated concentration of 4 x 10^4 cfu/g feed. The feeds were subsequently treated with formaldehyde (660 mg/kg). After 24 hours the feeds were analysed for bacterial counts and formaldehyde content. *E. coli*-numbers in the untreated and treated feeds are given in Table 3.

It can be seen, that there is a decrease of 1 – 4 logs in the *E. coli* counts between the untreated and treated feeds. However, there were wide unaccounted differences in the level of original bacterial counts in the feeds. In several samples the levels of *E. coli* were much lower than the intended inoculum size (approximately log 4.6 cfu/g feed). Apparently no attempt was made to type the *E. coli* recovered from the feed and to demonstrate its identity as the one that was inoculated. There were also fluctuations in the amount of formaldehyde recovered, some apparent recoveries being higher than the dose originally applied.

Table 3. *E. coli* counts in artificially contaminated feeds untreated or treated with formaldehyde (660 mg/kg)

Type of feed	log *E. coli* cfu/g)		Formaldehyde recovered (mg/kg)
	Untreated	Treated	
broiler starter	3.39	< 1	526
broiler grower	3.32	< 1	362
broiler finisher	4.10	< 1	690
breeder mash	3.33	< 1	720
chick starter	5.46	4.65	486
pullet grower	2.66	< 1	478
layer mash	5.40	3.37	504
pig weaner	5.52	3.64	537
pig grower	3.55	2.00	582
pig finisher	3.31	1.48	557
sow breeder	2.22	1.08	467

In the second study the feeds were contaminated either with *E. coli* ATCC 31617 (serotype 0101:K30:K99:NM) or *Salmonella typhimurium* (ATCC 14028). The final inclusion rate for *E. coli* was approximately 10^3 and for *S. typhimurium* 10^4 cfu/g final feedingstuff. Otherwise the experimental protocol was that used in the first experiment. The bacterial counts in treated and untreated feeds are given in Table 4.

Table 4. *E. coli* and *Salmonella* counts in artificially contaminated feeds untreated or treated with formaldehyde (660 mg/kg)

Type of feed	Formaldehyde (mg/kg)	log *E. coli* (cfu/g)		log *Salmonella* (cfu/g)	
		Untreated	Treated	Untreated	Treated
Pig weaner	691.9	3.65	2.41	4.55	2.16
Pig grower	554.6	3.47	2.49	4.41	2.45
Pig finisher	653.3	3.38	2.06	4.65	2.08
Sow breeder	542.3	3.61	2.35	4.56	2.74
Poultry breeder	545.9	4.11	1.95	3.79	2.44
Broiler starter	608.4	3.71	1.77	4.38	1.76
Broiler grower	638.5	3.55	< 0	4.66	2.53
Broiler finisher	644.2	3.45	< 0	4.64	2.53
Chick starter	608.4	3.33	< 1	4.72	2.16
Puller grower	617.1	3.25	2.16	4.60	2.11
Layer mash	590.8	3.44	2.31	4.76	1.87

In this experiment the decrease in bacterial counts in the treated samples compared to the untreated ones was 1 – 4 log cycles. The variation in bacterial counts was less prominent than in the first experiment and the differences in *E. coli* and *Salmonella* numbers corresponded roughly to the respective sizes of the inocula.

5.3. Conclusions on efficacy

The data presented in the original dossier and in the resubmitted one demonstrate that formaldehyde has a certain antimicrobial action against *E. coli* and *Salmonella*. Despite deficiencies in the quality of data, especially in the first artificial contamination study cited above, it can be concluded that this effect is present in feeds of variable composition at the proposed inclusion rate (660 mg/kg).

6. SAFETY FOR THE TARGET ANIMALS

6.1. The effect of the recommended dose on piglets

Since the original dossier was presented in 1993 and the additional data submitted in 1996, an additional trial (1999) was made with pigs to monitor the toxicity of formaldehyde at the recommended application rate.

Early weaned piglets with 6 kg body weight were randomly allocated to two structurally identical but separate units on the farm for 154 days. One unit housed piglets given the control untreated feed and the second unit housed piglets given feed treated with 660 mg formaldehyde/kg feed. Each unit housed 1500 piglets. Piglets were housed in pens with 30 piglets in each pen.

There was no significant difference in total feed consumed, final weight and feed to gain ratio between pigs given the control untreated feed or those given feed treated with 660 mg formaldehyde/kg feed (Table 5).

Table 5: Effect of the treatment of feed with formaldehyde on pigs ($P<0.05$)

Formaldehyde dosage, mg/kg feed	Total feed consumed, kg	Mean final live weight, kg	Feed conversion, kg/kg
0	274.3	116.6	2.48
660	275.2	115.2	2.52

Macroscopic and microscopic examination of heart, liver, kidney, stomach and intestines was conducted on five animals per group. No significant lesions, necrosis, fibrosis or abnormal inflammation were observed in any of the tissues. No evidence of significant infectious, degenerative or toxic disease processes was observed. Focal areas of lymphocyte infiltration were noted in the mucosa of the stomach of two pigs of the control group and four piglets of the treated group. Focal areas of lymphocyte infiltration were noted in the interstitium of the kidney of 3 pigs in the treated group.

6.2. Tolerance tests on piglets

A first feeding experiment used 32 piglets (24.7 $\pm$0.315 kg body weight) in four groups receiving 0, 330, 990, 3300 mg formaldehyde/kg feed for 21 days. A second feeding experiment used 40 piglets (33.6 $\pm$0.445 kg body weight) in four groups receiving 0, 660, 1990, 6600 mg formaldehyde/kg

feed for 21 days. The piglets were paired according to their live weight and one pair of piglets was allocated to each pen.

No difference on performance expressed as body weight gain, feed intake and feed to gain ratio were recorded between the control group and groups receiving 330, 990, 3300 mg formaldehyde/kg feed in experiment 1 (Table 6).

In the second experiment, there was no effect on weight gain, feed intake and feed to gain ratio with application rates up to three times the recommended rate. At the end of the second trial, the piglets were slaughtered and gross pathology and organ weights noted. However, at ten times the recommended rate, the daily feed intake was reduced by approximately 40 %, which resulted in a reduction in daily live-weight gain and an increase in feed to gain ratio (Table 6).

Table 6: Effect of the treatment of feed with formaldehyde of piglets (mean ±SE)

Experiment No.	Formaldehyde dosage, mg/kg feed	Weight gain, kg/pig/day	Feed intake, kg/day	Feed conversion, kg feed/kg weight gain
1	0	0.41 ±0.02	1.35 ±0.124	3.26 ±0.14
	330	0.43 ±0.02	1.38 ±0.071	3.26 ±0.15
	990	0.41 ±0.05	1.39 ±0.168	3.45 ±0.29
	3330	0.37 ±0.02	1.27 ±0.074	3.49 ±0.13
2	0	0.56 ±0.06	1.97 ±0.11	3.57 ±0.02
	660	0.63 ±0.02	2.06 ±0.05	3.57 ±0.10
	1990	0.55 ±0.05	2.11 ±0.08	3.93 ±0.24
	6600	0.19 * ±0.03	1.16* ±0.08	6.44* ±0.80

*P<0.01

There was no significant difference in relative organ weights of any of the groups of piglets for any of the organs studied (Table 7). No abnormalities were recorded in any of the carcasses of the piglets.

Table 7: Effect of the treatment on organ weight

Experiment No.	Formaldehyde dosage, mg/kg feed	Relative organ weight (mean ±SE)		
		Liver	Kidney	Spleen
2	0	22.67 ±0.99	4.03 ±0.10	1.67 ±0.07
	660	21.33 ±0.72	3.95 ±0.12	1.97 ±0.11
	1990	21.12 ±0.95	3.84 ±0.15	1.79 ±0.11
	6600	20.04 ±0.63	3.78 ±0.19	1.71 ±0.10

6.3. Conclusions on tolerance of piglets

These two tests demonstrated that pigs tolerated application of formaldehyde to feed at three times the recommended application rate, however at 10x this concentration the feed was unpalatable and feed intake was reduced. These tests on piglets were conducted only over a period of 21 days, instead of 30 days, currently required by SCAN. In the study using the normal feeding

level, some increase in inflammatory infiltrations in stomach and kidney were reported. In the absence of further data, it cannot be excluded that these effects were caused or influenced by the presence of formaldehyde in the feed.

6.4. Tolerance tests on broilers

Three experiments were carried out with broilers offered untreated feed or feed treated with formaldehyde.

Trial 1

In this study 128 broilers were distributed to four groups receiving 0, 375, 1125, 3750 mg formaldehyde/kg feed for 21 days. No adverse affects on health or performance were observed at the 1125 mg formaldehyde treatment level. At the 3750 mg formaldehyde/kg feed treatment level, formaldehyde caused a decrease in body weight and performance. Histopathological examination revealed an irritation of the mucosal lining of the proventriculus and ventriculus.

Trial 2

In test 2, 160 broilers were divided into five groups each receiving 0, 660, 1980, 3300, or 6600 mg formaldehyde/kg feed for 30 days. There were four replicates per treatment group in the weeks 1, 2 and 3 and three replicates in week 4. Each replicate comprised 8 broilers. At 30 days of age all broilers were killed and organ weights were noted.

The feed consumption of all formaldehyde treatment groups was significantly different from the control at the age of 1 and 2 weeks and remained so for the 1980 mg, 3300 mg and 6600 mg formaldehyde treatment groups throughout the trial. However, in weeks 3 and 4 no significant differences in intake were found between control and the 660 mg group. A significant decrease in total feed consumption was observed for the three highest treatment groups but not for the group given feed treated at the recommended application rate (Table 8) .

Table 8: Effect of formaldehyde on feed consumption

Formaldehyde dosage, mg/kg feed	Feed consumption (g/broiler/week)				
	Week 1	Week 2	Week 3	Week 4	Total
0	172.8 [a]	330.8 [a]	624.8 [a]	667.9 [a]	1797.0 [a]
660	151.4 [b]	295.9 [b]	594.7 [a]	656.2 [a]	1721.1 [a]
1980	139.9 [c]	282.7 [b]	537.0 [b]	634.9 [b]	1577.9 [b]
3300	131.9 [c]	242.7 [c]	419.4 [c]	489.8 [b]	1306.4 [c]
6600	94.3 [d]	121.6 [d]	167.1 [d]	173.0 [c]	562.2 [d]

[a,b,c,d] – Values in columns with different superscripts differ significantly ($P<0.05$)

The body weight of the 660 mg formaldehyde treatment group was not significantly different from the control at the age of 1, 3 and 4 weeks but was significantly reduced at the age of 2 weeks. From 1 week onwards the body weight of the birds in the three highest treatment groups was significantly

lower compared to those in the control group and in the group given feed treated at the recommended application rate (Table 9).

Table 9: Effect of formaldehyde on body weight

Formaldehyde dosage, mg/kg feed	Body weight (g/broiler)				
	Week 0	Week 1	Week 2	Week 3	Week 4
0	45.7	164.8 [a]	375.8 [a]	775.3 [a]	1029.0 [a]
660	45.5	154.9 [a]	345.7 [b]	730.5 [a]	1003.8 [a]
1980	46.1	138.0 [b]	288.2 [c]	595.4 [b]	830.8 [b]
3300	46.9	125.9 [c]	236.3 [d]	432.6 [c]	595.8 [c]
6600	47.4	91.7 [d]	126.2 [e]	186.2 [d]	220.2 [d]

[a,b,c,d] – Values in columns with different superscripts differ significantly ($P<0.05$)

The feed to gain ratio recorded for the 1980 mg, 3300 mg and 6600 mg formaldehyde treatment groups differed significantly from the control group throughout the trial (Table 10).

Table 10: Effect of formaldehyde on feed to gain ratio

Formaldehyde dosage, mg/kg feed	Feed conversion (g feed/g body weight gain)				
	Week 1	Week 2	Week 3	Week 4	Cumulative
0	1.45 [a]	1.57 [a]	1.57 [a]	2.52 [a]	1.82 [a]
660	1.39 [a]	1.55 [a]	1.56 [a]	2.67 [a, b]	1.80 [a]
1980	1.52 [a]	1.88 [b]	1.75 [a]	2.81 [a, b]	2.01 [b]
3300	1.68 [b]	2.20 [c]	2.14 [b]	3.13 [b]	2.39 [c]
6600	2.14 [c]	3.51 [d]	2.94 [c]	5.08 [c]	3.27 [d]

[a,b,c,d] – Values in columns with different superscripts differ significantly ($P<0.05$)

In the 660 mg and 1980 mg treatment groups, there were no significant differences in organ weights relative to body weight when compared to the control (Table 11).

Table 11: Effect of formaldehyde on relative organ weight

Formaldehyde dosage, mg/kg feed	Relative organ weight (g/100g body weight)						
	Liver	Spleen	Pancreas	Heart	Bursa	Proventriculus	Ventriculus
0	2.83 [a]	0.14	0.25 [a,b]	0.56 [a,b]	0.26 [a,b]	0.76 [a]	1.96 [a]
660	2.46 [a]	0.13	0.22 [a]	0.52 [a]	0.25 [a,b]	0.75 [a]	1.83 [a]
1980	3.06 [a,b]	0.15	0.26 [a,b]	0.62 [b,c]	0.31 [a,c]	0.95 [a, b]	1.91 [a]
3300	3.57 [b]	0.16	0.27 [b]	0.68 [c]	0.35 [c]	1.10 [b]	2.22 [a]
6600	3.57 [b]	0.14	0.32 [c]	0.94 [d]	0.24 [b]	1.03 [b]	3.25 [b]

[a,b,c,d] – Values in columns with different superscripts differ significantly ($P<0.05$)

During the post-mortem examination, organs were macroscopically examined for abnormalities. In the 3300 mg formaldehyde treatment group the lining of the ventriculus from one broiler was observed to be irritated. This irritation did not appear to penetrate the lining to the muscular tissue. In the 6600 mg formaldehyde treatment group three broilers were observed to exhibit a similar irritation of the ventricular lining.

Trial 3

In the third study 160 broilers were used in five groups receiving 0, 660, 1980, 3300, 6600 mg formaldehyde/kg feed for 28 days. There were four replicates per treatment; a replicate comprised 8 broilers. At 28 days of age all broilers were killed and organ weights were recorded.

The feed consumption of the 660 mg formaldehyde treatment group was higher and significantly different from the control group at the age of 2 weeks but not thereafter. The feed consumption of the 1980 mg formaldehyde treatment group did not differ significantly from that of the control group (Table 12).

Table 12. Effect of formaldehyde on feed consumption

Formaldehyde dosage, mg/kg feed	Feed consumption (g/broiler/week)				
	Week 1	Week 2	Week 3	Week 4	Total
0	167.0 ^{a,b}	398.7 ^{a}	630.8 ^{a}	716.5 ^{a,b}	1913.0 ^{a}
660	174.6 ^{a}	436.6 ^{b}	658.7 ^{a}	767.3 ^{a}	2037.0 ^{a}
1980	163.9 ^{b}	373.4 ^{a}	603.9 ^{a}	770.1 ^{a}	1911.2 ^{a}
3300	141.6 ^{c}	302.9 ^{c}	462.9 ^{b}	605.5 ^{b}	1512.9 ^{b}
6600	81.4 ^{d}	122.3 ^{d}	122.1 ^{c}	151.1 ^{c}	477.0 ^{c}

^{a,b,c,d} – Values in columns with different superscripts differ significantly ($P<0.05$)

The body weight of birds in the 660 mg formaldehyde treatment group did not differ significantly from those in the control group throughout the trial. In the 1980 mg formaldehyde treatment group, bodyweight was different from the control group at 1,2 and 3 weeks of age, but was not significantly different at 4 weeks of age. In the 3300 mg and 6600 mg formaldehyde treatment groups, body weight was reduced significantly compared to the controls by the end of the first week and this continued throughout the trial resulting in a very substantial reduction by the forth week (Table 13).

Table 13. Effect of formaldehyde on body weight

Formaldehyde dosage, mg/kg feed	Body weight (g/broiler)				
	Week 0	Week 1	Week 2	Week 3	Week 4
0	43.1 ^{a,b}	166.4 ^{a}	419.2 ^{a}	786.2 ^{a}	1151.3 ^{a,b}
660	43.4 ^{a}	172.2 ^{a}	434.8 ^{a}	816.9 ^{a}	1235.2 ^{a}
1980	41.3 ^{b}	145.5 ^{b}	347.7 ^{b}	661.1 ^{b}	1068.3 ^{b}
3300	42.6 ^{a,b}	108.2 ^{c}	218.9 ^{c}	386.9 ^{c}	632.7 ^{c}
6600	41.7 ^{a,b}	65.0 ^{d}	85.2 ^{d}	112.5 ^{d}	146.9 ^{d}

^{a,b,c,d} – Values in columns with different superscripts differ significantly ($P<0.05$)

In the 3300 mg and 6600 mg formaldehyde treatment groups, the decrease in body weight was associated with a significant decrease in feed consumption and this resulted in a significant increase ($P<0.05$) in feed conversion (Table 14).

Table 14. Effect of formaldehyde on feed conversion

Formaldehyde dosage, mg/kg feed	Feed conversion (g feed/g body weight gain)				
	Week 1	Week 2	Week 3	Week 4	Cumulative
0	1.36 [a]	1.58 [a]	1.72 [a]	2.00 [a]	1.73 [a]
660	1.36 [a]	1.67 [a]	1.73 [a]	1.89 [a]	1.72 [a]
1980	1.57 [b]	1.85 [a]	1.93 [a]	1.89 [a]	1.86 [a]
3300	2.16 [c]	2.74 [b]	2.76 [b]	2.46 [b]	2.56 [b]
6600	3.50 [d]	6.14 [c]	4.52 [c]	4.38 [c]	4.55 [c]

[a,b,c,d] – Values in columns with different superscripts differ significantly ($P<0.05$)

In the 660 mg group, there were no significant differences ($P>0.05$) in organ weights relative to body weight when compared to the control. In the 1980 mg treatment group the pancreas weight and ventriculus weight relative to body weight and in the 3300 mg and 6600 mg treatment group the pancreas, heart, bursa, proventriculus and ventriculus weights relative to the body weight were significantly heavier than in the control group (Table 15).

During the post-mortem examination, organs were macroscopically examined for abnormalities. Abnormalities were found in one bird from the 1980 mg treatment group, three birds from the 3300 mg and four birds in the 6600 mg treatment groups, the lining of the ventriculus was observed to be irritated. This irritation did not appear to penetrate the lining to the muscular tissue. Slight irritation of the ventriculus was also observed in one bird from the control group.

Table 15. Effect of formaldehyde on relative organ weight

Formaldehyde dosage, mg/kg feed	Relative organ weight (g/100g body weight)						
	Liver	Spleen	Pancreas	Heart	Bursa	Proventriculus	Ventriculus
0	2.53 [a]	0.13 [a,b]	0.22 [a]	0.62 [a]	0.22 [a]	0.50 [a]	1.68 [a]
660	2.57 [a]	0.14 [a]	0.22 [a]	0.58 [a]	0.21 [a]	0.50 [a]	1.74 [a,b]
1980	2.51 [a]	0.13 [a,b]	0.24 [b]	0.63 [a]	0.23 [a]	0.55 [a]	1.88 [b]
3300	2.71 [a]	0.11 [b]	0.27 [c]	0.75 [b]	0.27 [b]	0.70 [b]	2.33 [c]
6600	2.81 [a]	0.08 [c]	0.33 [d]	1.14 [c]	0.17 [c]	0.91 [c]	3.94 [d]

[a,b,c,d] – Values in columns with different superscripts differ significantly ($P<0.05$)

6.5. Conclusions on tolerance of broilers

In these tolerance tests on growing chickens a dose dependent decrease in feed intake and body weight gain was noted when formaldehyde is added at levels 1980 mg, 3300 mg and 6600 mg. In trial 2 an indication of reduced feed intake and body weight gain during the first trial weeks could be seen even at the recommended dose level of 660 mg/kg feed.

In test 2 and in test 3 there is a general decrease in the absolute weights of the organs that were weighed. Relative weights of most of the organs increased. The exception to this was an decrease in relative weights of spleen and bursa at 6600 ppm only. This observation may reflect toxicity.

Although chickens for fattening were able to tolerate feed treated with formaldehyde at the recommended application rate there was little margin for error before adverse effects were apparent

7. USER SAFETY

7.1. Types and extent of exposure

Worker exposure may occur as a result of skin contact with formaldehyde or the treated feed. Workers may also be exposed by inhalation of formaldehyde vapour or of dust from the treated feed. Exposure may take place whilst the formaldehyde is being mixed into the feed or to a lesser extent when handling the treated feed.

Feed treated with formaldehyde at the maximum recommended amount would contain 660 mg/kg of total formaldehyde. Such treated feed had an initial concentration of 36.5 mg/kg of free (ie. unbound) formaldehyde immediately after mixing. The concentration of free formaldehyde reduced on storage to 22.9 mg/kg after 1 hr. There was some loss of formaldehyde due to volatilisation, with 5.53% of the 660 mg/kg of formaldehyde that was added to a feed being lost after 24 hrs of storage under forced ventilation conditions.

7.2. Sensitising properties and irritancy

The formaldehyde is applied to feed as an aqueous solution (formalin). Formaldehyde caused mild to moderate skin irritancy in rabbits and guinea-pigs when it was tested in aqueous solutions at concentrations of 0.1% to 20% (1000 - 200 000 mg/kg). It will need to be labelled as irritant to eyes and skin, in line with the labelling rules applied to other presentations of formalin (eg use in hospital laboratories or in the chemical industry).

There is clear evidence that high concentrations (400 mg/kg or more in aqueous solution) of formaldehyde can induce skin sensitisation in humans. In addition 11 out of 18 formaldehyde-sensitive people reacted to 100 mg/kg of formaldehyde in skin creams. As no clear threshold has been identified for induction of sensitisation or for production of adverse effects in sensitised individuals, it is not clear whether exposure to the free formaldehyde remaining in the treated feed will be harmful to operators. It is also unclear whether bound formaldehyde has the potential to cause sensitisation.

Formaldehyde does not meet the European requirements for classification as a respiratory sensitiser.

7.3. Occupational safety requirements

There is wide experience of the use of formaldehyde in many industries throughout the European Community. Individual member states have set occupational exposure limits for formaldehyde, covering short-term exposure and longer exposure. These range from 0.4 mg/m^3 (0.3 ppm) in

Denmark to 3.0 mg/m^3 (2.4 ppm) in France & the Netherlands for 15 minute short-term exposure limits (STELs); and range from 0.6 mg/m^3 (0.5 ppm) in Germany & Sweden to 2.5 mg/m^3 (2.0 ppm) in the UK for an 8 hr time-weighted average (TWA) exposure. No EU-wide occupational exposure limits are in place.

Air concentrations in the vicinity of farm workers were measured by personal monitors whilst treated feed was being handled. The feedstuff had been treated with an aqueous solution of 37% formaldehyde at a rate of 1 kg formaldehyde/ton of feed (330 mg/kg). Measurements were taken at 4 & 24 hrs after the feed had been treated with formaldehyde. The air concentrations measured over a 15 minute monitoring period were between 0.09 and 0.26 ppm for the feed treated 4 hrs previously. No formaldehyde was detected in the air whilst handling the feed that had been treated 24 hrs previously. Whilst the highest of these figures was just below the lowest of the national occupational exposure limits, it is noted that the amount of formaldehyde used for treating the feedstuff in this experiment was only half of the maximum permitted amount. It seems likely that the STELs of at least some member states would be exceeded if feed treated with the maximum permitted amount of formaldehyde was to be used soon after treatment.

Measurements of air concentrations of formaldehyde taken at feed mills whilst the feed was being treated with formaldehyde showed levels of up to 0.71 ppm. However, in 90 out 109 samples the concentration was below the limit of detection of 0.05 ppm. These results indicate that there is potential for the STELs of at least some Members States to be exceeded at feed mills.

7.4. Conclusions

Since there are no specific threshold levels for formaldehyde sensitisation of skin and no data on the skin sensitising potential of bound formaldehyde, formaldehyde and the feed should be treated as skin sensitisers.

As a precaution, treated feed should not be handled on the farm until at least 24 hrs after treatment.

The air concentrations of formaldehyde around workers handling treated feed exceed the occupational exposure limits of some EU Member States. This may require appropriate risk management measures.

8. CONSUMER SAFETY

8.1. Formaldehyde residues in animal tissues

Formaldehyde is a naturally occurring compound in animal tissues. The company has provided data on formaldehyde levels in animal products derived from poultry and pigs receiving either formaldehyde-treated or untreated feed. The analytical method in these studies was based on the spectrophotometric measurement of the colour reaction between tissue distillate and Hantz reagent. According to the results formaldehyde at the

level of 660 mg/kg feed does not cause a detectable increase in the tissue levels of formaldehyde (Tables 16, 17 and 18).

Table 16. Levels of formaldehyde in the eggs and tissues of laying hens receiving either formaldehyde-treated or untreated feed for at least 30 weeks.

Poultry eggs / tissue	Untreated feed				Feed treated with formaldehyde (660 mg/kg)			
	mg formaldehyde/kg fresh tissue				mg/formaldehyde/kg fresh tissue			
	Number of samples	Mean	SD	95% confidence limits	Number of samples	Mean	SD	95% confidence limits
Eggs	70	19.9	14.72	16.54 – 23.43	46	7.9	6.39	6.11 – 9.79
Muscle	30	6.6	4.6	4.96 – 8.25	35	8.3	5.37	6.48 – 10.03
Liver	30	8.2	4.41	6.66 – 9.82	38	9.6	6.24	7.66 – 11.62
Kidney	19	37.1	30.45	23.77 – 50.45	25	29.1	23.49	20.05 – 38.09
Fat/skin	20	56.5	50.59	34.81 – 78.09	10	35.4	11.35	28.72 – 42.12

Table 17. Levels of formaldehyde in tissues of piglets fed with either formaldehyde-treated or untreated feed from weaning to the age of 10 weeks (20 – 30 kg)

Pig tissue	Untreated feed				Feed treated with formaldehyde (660 mg/kg)			
	mg formaldehyde/kg fresh tissue				mg/formaldehyde/kg fresh tissue			
	No. of samples	Mean	SD	95% confidence limits	No. of samples	Mean	SD	95% confidence limits
Muscle	3	35.3	2.60	32.7 – 37.9	3	28.3	1.69	26.6 -29.9
Liver	3	25.4	3.17	22.2 – 28.6	3	23.8	1.36	22.4 – 25.2
Kidney	3	29.1	2.60	26.5 – 31.7	3	23.0	3.18	19.8 – 26.2
Fat/skin	3	25.9	2.94	23.1 – 28.9	3	29.5	2.15	27.4 – 31.7

Table 18. Levels of formaldehyde in tissues of slaughter pigs (110 – 120 kg)[1] fed for 18 weeks with either with untreated feed or feed treated with formaldehyde.

Treatment	Formaldehyde concentration in tissue (mg/kg)			
	Muscle	Liver	Kidney	Fat
Untreated	0.42 ± 0.09	< 0.2	< 0.2	0.85 ± 0.17
Formaldehyde (660 mg/kg)	0.34 ± 0.05	< 0.2	< 0.2	0.17 ± 0.14

[1] No indication of the actual number of pigs used in the study was given

8.2. Conclusions

In the reported studies there is no indication of increased consumer exposure to formaldehyde due to eating animal products from target species that had been given feed treated with formaldehyde at the proposed inclusion rate (660 mg/kg).

9. ENVIRONMENTAL SAFETY

9.1. The amount of formaldehyde residues in pig and poultry faeces

The company has analysed the faecal content of formaldehyde from pigs and poultry given feedingstuffs treated with formaldehyde. The analysis was done using two different methods: the Hantz-reagent based method that was also used in tissue residue study, and chromatrophic acid-sulphuric acid reaction. The latter detects mainly free formaldehyde and was the method of choice for measuring airborne pollution, but was, in this case, applied to the faecal distillate that contains the formaldehyde liberated from faecal matter.

In a trial with 20 – 30 kg pigs that had for six weeks received feed treated with variable amounts of formaldehyde, there was a clear dose dependent correlation between the faecal formaldehyde content and the level of formaldehyde in the feed (Table 19. The analysis was performed using the Hantz-reagent.

Table 19 The faecal formaldehyde levels of pigs that had consumed feed treated with formaldehyde at levels ranging from 330 – 990 mg/kg

Formaldehyde in feed (mg/kg)	Number of animals	Formaldehyde in faeces (mg/kg)
0	5	0.35 ± 0.02
330	5	1.22 ± 0.02
660	5	2.78 ± 0.25
990	5	4.43 ± 0.57

In another two experiments the method used for analysis was based on chromatrophic-sulphuric acid reaction. The pigs were either 11 week old (40 kg), or 20 weeks old (110kg). The corresponding feeding periods were 9 and 18 weeks, and the level of formaldehyde in the treated feed 660 mg/kg.. Again, there was distinctly more formaldehyde in the faeces of pigs receiving the treated feed. However, the concentrations were distinctly lower than in the first study (Table 20)

Table 20. The faecal formaldehyde levels of pigs that had consumed feed treated with formaldehyde at the level of 660 mg/kg.

Formaldehyde in feed (mg/kg)	No. of animals	Faecal formaldehyde (mg/kg)	
		40 kg pigs	110 kg pigs
0	5	0.07 ± 0.01	not detectable
660	5	0.22 ± 0.05	0.47 ± 0.08

The formaldehyde analyses in poultry excreta were performed using litter samples of poultry houses containing 25,000 – 30,000 birds each. Six samples were taken and pooled from each house. At the time of sampling the birds were approximately. 33 days of age, and had been fed continuously either formaldehyde-treated (660 mg/kg) or untreated feed. Analysis was based on Hanz-reagent. The formaldehyde levels in the litter were distinctly

higher in the poultry houses where the birds had received formaldehyde-treated feed (Table 21)

Table 21. Formaldehyde levels in litter samples from poultry houses with birds having been reared with feed either treated or untreated with formaldehyde (660 mg/kg) for 33 days.

Formaldehyde in the feed (mg/kg)	No of houses	Formaldehyde in the litter (mg/kg)
0	2	0.006 ± 0.003
660	9	5.8 ± 0.4

The low value reported above is in contrast to the considerably higher mean value of 42.4 mg/kg reported from the 30-day formaldehyde tolerance study on broilers at the dose level of 660 mg/kg (trial 2). The latter value is more in agreement with the values obtained from three studies on layer hens exposed to formaldehyde treated feed (660 mg/kg) for longer periods than 30 – 33 days (Table 18).

Table 18. Formaldehyde in the litter from layer hens fed with formaldehyde –treated feed (660 mg/kg feedingstuff)

Duration of the feeding (weeks)	Formaldehyde in the litter (mg/kg)
39[1]	43.35
47[2]	33.0
57[1]	46.8

[1] Analyses based on Hanz-reagent
[2] The analytical method not mentioned.

9.2. The emission of formaldehyde from the excreta

The company has monitored the amounts of formaldehyde emitting from the poultry litter samples using the Gastec Passive Dosi-tube (No 91D). The samples were collected from seven poultry houses. The birds were 69 weeks of age and had received formaldehyde-treated feed (660 mg/kg) for 57 weeks.

No emission of formaldehyde was registered, the limit of detection being 0.05 ppm.

9.3. The effects of formaldehyde on different life forms

The Company has collected a large number of data on the sensitivities of algae, bacteria, water-flea, crustaceans, molluscae, and various species of fish to environmental formaldehyde. While the LC_{50} values for algae are rather low (0.3 – 0.4 mg/l) most other organisms tolerate higher formaldehyde concentrations. The LC_{50} values for fishes vary between 11.8 mg/l (striped bass) to 105 mg/l (largemouth bass).

There are apparently few studies on the effects of formaldehyde on plants. The few experiments that have been reported deal either with the effects of

atmospheric formaldehyde or aqueous formaldehyde solutions applied directly on plants or on soil and thus are of little relevance regarding bound formaldehyde in animal excreta.

9.4. The breakdown of formaldehyde in the environment

The company refers to documents published by National Research Council (1981a) and a subsequent WHO opinion (1989) on the rapid degradation of formaldehyde in air and water with a calculated half life of few hours in the air. A Japanese study (Kamata 1966) is also cited showing aerobic decomposition of formaldehyde in water to occur in ca. 30 h while in anerobic conditions the process required ca. 48 h. There are also numerous studies showing that several bacterial species, including those found in activated sludge, can utilise formaldehyde.

9.5. Conclusions

Although the different analytical methods and variable experimental conditions applied make the evaluation of data on formaldehyde residues in target animal excreta difficult, it is apparent that formaldehyde levels are elevated as a result of formaldehyde treatment of feeds. However, the formaldehyde appears to be in bound form limiting its release to environment.

Most aqueous organisms seem to tolerate relatively high concentrations of formaldehyde, and thus keeping in mind its rapid degradation, formaldehyde in the excreta does not present an environmental problem.

10. GENERAL CONCLUSIONS

10.1. Efficacy

On the basis of the data supplied and in view of the proposed level of use in the feed, SCAN considers formaldehyde rather as a disinfectant than a preservative agent.

As already stated in its previous opinion of 1999, a single application of formaldehyde at the proposed concentration can reduce the bacterial numbers in the feed. This effect apparently occurs in several types of feeds and raw materials artificially contaminated with *Salmonella* and *E. coli*.

However, without a clear claim concerning the intended use, and because no studies have been reported showing actual reduction of *Salmonella* spp. in animals or animal products as a result of the use of formaldehyde, it is difficult to conclude anything more specific about its efficacy.

10.2. Safety of target animals

The tolerance tests on piglets did not show any adverse effects at doses up to three times the recommended level. However at the recommended dose, adverse effects at the level of histopathology cannot be excluded. In addition, these tests were conducted only over a period of 21 days and not the minimum of 30 days, currently required by SCAN.

For the broilers, the safety margin appears to be very narrow, adverse effects being apparent at a dose level of three times the recommended dose. In one of the reported tolerance tests some indication of decreased feed intake and weight gain was observed even at the recommended dose.

SCAN therefore concludes that the safety for both target animal species has not been unequivocally demonstrated.

10.3. User safety

The concentration of formaldehyde used is this product is irritant for skin and mucous membranes. Formaldehyde is also a sensitiser. Appropriate management measures should be taken accordingly.

10.4. Consumer safety

There is no indication that the levels of formaldehyde in the animal product derived from the target species would exceed the endogenous levels as a result of formaldehyde treatment of feeds at the proposed dose level. Thus there appears to be no concern of consumer safety.

10.5. Environmental safety

The use of formaldehyde in feeds leads to increased formaldehyde levels in pig and poultry excreta. However, as formaldehyde is both chemically and

biologically rapidly degraded in the environment, there appears to be no increased environmental risk associated with the intended use.

11. REFERENCE

Reply by Anitox Ltd to complementary information requested (June 2001) by the Scientific Committee on Animal Nutrition on the use of formaldehyde as a preserving agent for pig and poultry feedingstuff (November 2001)