REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF METHYLPROPIONIC (ISOBUTYRIC) ACID IN FEEDINGSTUFFS

(Opinion expressed: March 1993)

TERMS OF REFERENCE (November 1985):

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

1. Is the use of methylpropionic (isobutyric) acid as a preservative at the level of 0.2%-0.6% in complete feedingstuffs safe for target species, in particular for poultry?
2. Should it be necessary to discard this product from feedingstuffs for dairy cattle because of a possible adulteration of the taste of milk?
3. Does the proposed use result in residues in animal produce which could be prejudicial to the consumer?

BACKGROUND:

In 1985 an opinion of SCAN was requested to establish whether the use of methylpropionic (isobutyric) acid (MPA) under the proposed conditions was in conformity with the requirements of Council Directive 70/524/EEC, of 23 November 1970, concerning additives in feedingstuffs [1]. This preservative for feedingstuffs has been listed in Annex II of Council Directive 70/524/EEC as amended by Council Directive 84/587/EEC[2] for the past 5 years and a decision is now required regarding transfer of MPA to Annex I or deletion.

In December 1983 doubts had been expressed over the safety of MPA, particularly over two aspects of the then available data :

1. a dose-dependent increase in liver weight had been observed in both male and female rats fed MPA for 90 days and for which a NEL had not been established;

2. a dose-dependent decrease in the body weight of laying hens had been noted, which was already evident at doses comparable with the proposed feeding levels.

Additional information, received in February 1985, did not really allay those concerns. In its provisional opinion, dated 22 January 1986, SCAN requested further experiments to clarify the cause and nature of the liver enlargement observed in rats and the loss in body weight in layers. The Committee also thought that the use of MPA in feedingstuffs for dairy cattle would not adversely affect the taste of the milk, nor would any residues of MPA in animal produce be prejudicial to the health of the consumer.

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1 O.J. No. L270 (04.12.70) p. 1

2 O.J. No. L319 (08.12.84) p. 13

The information received in November 1989 did not address the questions raised by the SCAN in January 1986. At its 70th meeting the SCAN rediscussed the hepatic effects of MPA and requested additional data on relevant serum enzyme levels as well as any other supporting evidence to resolve the problem of the hepatic enlargement.

No additional data have been received but the Committee has now been informed that the request for the use of MPA in feedingstuffs for laying hens has been abandoned.

OPINION OF THE COMMITTEE:

1. MPA is formed naturally in the stomach of monogastrics and in the rumen and is also present in faeces. It has antimicrobial and fungicidal properties but no data on its efficacy as a preservative of animal feedingstuffs have been provided (3).

   Concentrations of 0.05% are inhibitory or bactericidal for G-ve bacterial species, while concentrations between 0.05%-0.5% inhibit the growth of bacteria, yeasts and moulds, including species known to cause infections or to elaborate toxins. The likely reduction due to MPA in the intestinal microbial flora competing for the ingested food probably contributes a growth promoting effect. MPA is not used in human or veterinary medicine and exerts no therapeutic or preventive effects on animal disease (3).

   Tests to determine MIC, MBC and MFC have been carried out on G+ve bacteria, G-ve bacteria, yeasts and moulds.

   The effect on Salmonella excretion was tested in experimentally infected chicks. No evidence for a prolonged time of Salmonella excretion was found (3).

   Rats have been reported to absorb MPA nearly completely and chemically unchanged from the gastrointestinal tract and then to metabolise it in the liver.

   Methylmalonic acid and ß-hydroxybutyric acid appear as metabolites in the urine (4). MPA is known to be produced endogenously in mammals, including man, by the catabolism of L-valine. No specific data have been supplied on absorption, distribution and excretion of exogenously supplied MPA in the target species.

   In a 20-day feeding trial on laying hens, given up to 2% MPA in their feed, no adverse effects were noted on food intake, egg production and egg hatchability (3).

   A limited 3-months feeding study in pigs at dose levels up to 0.9% MPA in the diet produced no adverse effects on growth, haematology, absolute and relative organ weights (5 organs), and histopathology (32 organs examined) (3).

2. MPA, together with other volatile fatty acids, is produced in the rumen by microbial fermentation of branched-chain amino acids and completely absorbed. About 1000g butyric acid are absorbed daily and predominantly converted in the

rumen wall into ß-hydroxybutyric acid, a metabolite of MPA. This should be compared with the maximum absorption of 200g MPA from treated feedingstuff, thus representing about 10%-15% of the endogenous production. These amounts are too small to cause organoleptic changes in the milk (7). Feeding of ammonium isobutyrate to dairy cows did not increase the concentration of the free acid in the milk (8).

The addition of MPA to feedingstuffs of dairy cows has been shown not to increase the amount of free MPA in the milk and is therefore unlikely to affect the taste of the milk.

3. No residues were detected at any time in liver, kidney, muscle tissue, blood and fat of rats given a single oral dose of 400 mg MPA/kg b.w. (limit of detection 33 mg/kg)(3).

No residues were found in any egg of laying hens fed up to 2% MPA in their feedingstuff for 7 months (limit of detection 25mg/kg)(3).

No residues were found in the liver, kidney, muscle tissue, skin, fat and blood of pigs fed up to 0.9% MPA in their feedingstuff for 3 months (limit of detection 200 mg/kg)(3).

The tissues of other target animals were not examined for MPA residues. In general the methodology was rather insensitive. Residues of metabolites of MPA were not investigated.

The toxicity of MPA has been studied in acute, subacute, subchronic and chronic studies in the rat and chicken. The oral LD_{50} in the rat was 2.26g/kg b.w. and in the chicken 2.31g/kg b.w. No macroscopic pathology was noted (3). Female rats, fed up to 2% MPA in their feed for 42 days, showed no adverse effects on body weight gain, food intake or organ weights (10 organs). No haematology, clinical chemistry, urinalysis or histopathology were carried out as part of this study (3).

A 90-day feeding study in rats with up to 2% MPA in their feed showed clear evidence of hepatotoxicity. Sixteen day-old chicks, fed for 28 days up to 2.0% MPA in their feed, were said to show no adverse effects on body weight gain and haematology. Food conversion apparently improved dose-dependently. Original data were not supplied (3).

There was a dose-dependent loss in body weight in an in-feed toxicity study in chickens over and above that expected to occur on ageing. This became significant only at the highest dose level. No immunosuppressive action of MPA was noted. The NEL was 0.4% in the feed but the body weight loss has remained unexplained (3). No significant effects on egg production or egg quality was seen in another chronic feeding study extending over 364 days and particularly designed to study just this aspect (6).

As the request for the use of MPA in the feedingstuff for laying hens has now been withdrawn, the safety-in-use of MPA for laying hens is no longer an issue.

The genotoxicity of MPA was examined in an Ames test with/without activation. The test results were said to be negative but study details were not supplied. The meaningfulness of these results is doubtful in view of the known antimicrobial activity of MPA against G-ve bacteria (3). No tests have been carried out for point mutation or chromosomal effects in cultured mammalian cells (3).

A 4-generation reproduction study in rats with doses up to 2% MPA in the feed showed no consistent adverse effects related to the administration of MPA on parameters of reproductive function and survival and developmental parameters of the filial generations. No teratogenic effects were observed. Although a dose-related increase in liver weight was noted in some of the filial generations, no other parameters to assess the hepatotoxic significance of these findings were determined. No histopathological examinations were reported (3). Only brief summaries of 2 teratogenicity tests in rats with a negative outcome but lacking essential details for appraisal were reported (3).

A 10-day inhalation study in rats showed no significant irritant effects but also produced liver enlargement (3).

Although no specific studies on the environmental effects of MPA or its metabolites in excreted faeces and urine have been carried out, it is unlikely that these products would constitute a hazard for the environment. The faecal concentration of MPA after feeding 0.4% MPA in feedingstuffs was stated to be no greater than that produced by normal anaerobic bacterial metabolism (3).

MPA is an endogenously produced metabolite of L-valine catabolism in humans. Because of this, the rather insensitive method of analysis which demonstrated the absence of detectable residues in the carcases of rats and pigs and in eggs from laying hens, fed MPA in their feedingstuff, is nonetheless considered to exclude hazard to the consumer of products from animals given MPA-containing feedingstuffs.

References:

(3) Dossier submitted by Hüls AG.

(4) Baretz, B. & Tanaka, K. (1978) Metabolism in rats in vivo of isobutyrates labelled with stable isotopes at various positions. J.Biol.Chem., 253, 4203-4213.

(5) Danse, L.H.J.C. (1987) Letter to Dr. Rohte of 4.6.1987 SCAN/92/097.

(6) Tüller, R. & Velten, H.J. (1989) Report of 21.8.89 on effect of Meprosan on laying hens.

(7) Heeschen, W. (1985) Report on MPA effect on milk taste.

(8) Papas, A.M. & Sniffen, C.J. (1984) Effect of Feeding IsoC-4 and C-5 Volatile Fatty Acids as Ammonium Salts on Concentration of Free Volatile Fatty Acids in Milk. J.Dairy Sci., 67, 2887-2893.