Commission of the European Communities

agriculture

Reports of the Scientific Committee for Animal Nutrition

(Fifth series)

Report
EUR 10410 EN

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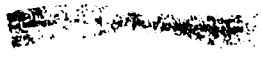

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FOREWORD

The fifth series of reports of the Scientific Committee for Animal Nutrition (1) contains the opinions expressed by the Committee in the period between 23 November 1983 and 26 September 1985. As in the past, the opinions are based on scrupulous evaluations involving research in many scientific fields and the very latest expert knowledge.

At the Committee's request, most of the additives were subjected to additional investigations deemed essential before any judgment could be pronounced. Detailed answers could thus be given to the various questions put by the Commission.

In addition, the Committee worked in close collaboration with the Scientific Committee for Food (2) to examine the effects of the use of protein products obtained by growing bacteria on methanol and yeast on alkanes for animal feeding. These studies and opinions provide essential information for the legislative authorities as well as being of topical interest to all who are concerned by the application of new biotechnologies in livestock production.

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(1) Previous series were published by the Office for Official Publications of the European Communities, Luxembourg, as follows :
1st series (1979) : Catalogue No CB-28-79-277
2nd series (1980) : No EUR 6918
3rd series (1981) : No EUR 7383
4th series (1984) : No EUR 8769

(2) The composition of the Committee was published in OJ No C 272, 11.10.1983, p. 2

COMPOSITION OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION (1)

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SECRETARIAT

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(1) Set up by the Commission Decision 76/791/EEC, 24.09.1976. (OJ No L 279, 9.10.1976, p. 35)
(2) Resigned from Committee on 10.09.1983
(3) Appointed on 23.12.1983
(4) Commission of the European Communities, Directorate-General for Agriculture

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SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF MONENSIN SODIUM IN FEEDINGSTUFFS FOR TURKEYS

Opinion expressed 23 November 1983

TERMS OF REFERENCE (July 1980)

In reply to questions put by the Commission on the safety of use of monensin sodium in feedingstuffs for turkeys, the Committee, in its report of 11 March and 9 December 1981 (*), considered that it could not express an opinion because of a lack of data on the metabolism of monensin in turkeys and on the nature and fate of its excretion products.

As the studies requested have been carried out and are now available, the Committee expressed the following opinion.

OPINION OF THE COMMITTEE

1. Tissue residues were determined in turkeys using monensin labelled with ^{14}C at seven specific sites. Birds were fed 110 mg labelled monensin/kg feed for five days and sacrified 6 hours after treatment, thus allowing equilibration of tissue levels at steady state turnover. Residues ranged from about 1 mg/kg in the liver to less than 0.2 mg/kg in kidney, skin and fat, and less than 0.05 mg/kg in muscle (limit of detection 0.03 mg/kg). These results are comparable to those found in chicken using similar ^{14}C-labelled material. The distribution of the metabolites in the liver and excreta was also examined and found to mimic the chicken data.

(*) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 25

Examination of the radioactive residues in the liver showed that about 1% of the total hepatic radioactivity was due to unchanged monensin, the remainder being distributed among the hepatic metabolities. Similarly, 7-8% of the total radioactivity in the excreta was represented by unchanged monensin, the rest being distributed among numerous faecal metabolites. The chromatographic pattern of the metabolites in faeces and liver appeared to be similar to that of the chicken, the main hepatic metabolite in both species being O-demethylated monensin. This compound has only 5% of the physiological activity of monensin.

Another residue study using turkeys fed for 17 weeks on 110 mg unlabelled monensin/kg feed gave results lower than those of the previous studies in liver, skin and kidney after 24 hours and no detectable residues after 48 hours. Analysis was carried out by microbiological assay (limit of detection 0.025 mg/kg tissue).

The use of monensin sodium up to the level of 110 ppm in the feedingstuffs for turkeys would thus not lead to any measurable residues in the treated birds, provided a withdrawal period of at least three days is observed.

2. A comparison of the studies using both ^{14}C-labelled and unlabelled monensin in chickens and turkeys reveals quantitative and qualitative similarities with regard to metabolite pattern and tissue residue levels. It may therefore be assumed that the data concerning the biodegradation of monensin and monensin metabolites from chicken excreta in soil and water are also valid for the same substances derived from turkey excreta.

Environmental effects were studied using excreta from chicken or steers. Chicken fed 120 ppm monensin in their feed for 90 days excreted between 1.2 - 3.6 ppm (wet basis) in the faeces. When chicken excreta were stored, the monensin content dropped to 15% within 4 days. The monensin content of manure from steers declined to 20% within 9 weeks and of faeces to 65% within 10 weeks.

Monensin degrades in soil within 4 weeks. It leaches only from sandy soils, 30% being recovered in the leachate. Monensin slowly degrades in water on exposure to light with a half-life of over 30 days.

Litter from broilers containing about 3 ppm was not phytotoxic to 14 plant species when applied at the rate of 10 tons/ha. Cattle manure containing 2.8 ppm monensin was not toxic to earth worms (Eisenia foetida). Concentrations of monensin up to 300 ppm produced no apparent toxicity in rainbow trout (Salmo gairdneri) treated for 14 days. The acute LD_{50} for bluegills (Lepomis macrochirus) was 33 ppm.

Monensin activity disappeared from activated sludge within 4 days and up to 25 mg/ml had no effect on the 5 day biochemical oxygen demand. Addition of pure monensin inhibits methane production in biogas production and also pure cultures of methanogenic bacteria. This activity is in agreement with the reduction in methane production observed in the rumen.

The use of monensin sodium in feedingstuffs for turkeys is thus not prejudicial to the environment.

3. On the basis of the available data, the Committee is of the opinion that the proposed conditions of use of monensin sodium in feedingstuffs for turkeys are acceptable, subject to a limitation of the maximum content to 110 mg/kg feedingstuff and provided a withdrawal period of at least 3 days is observed. However, the concentration resulting in maximum efficacy does not exceed 100 mg/kg.

REFERENCES

Dossiers supplied by Lilly Research Centre Ltd. (1983)

SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF NARASIN IN FEEDINGSTUFFS FOR CHICKENS

Opinion expressed 8 February 1984

TERMS OF REFERENCE (November 1980)

In reply to questions put by the Commission on the safety of use of narasin in feedingstuffs for chickens, the Committee, in its report of 14 April 1982 (*), considered that the proposed use could be admitted provisionally with a withdrawal period of at least five days before slaughter and that a reassessment would be envisaged when full data on the metabolism of narasin in chickens become available.

As additional studies have been carried out on the identity, the microbiological and biochemical activity of the major metabolites, and on the fate of tissue residues in chickens, the Committee expressed the following opinion.

OPINION OF THE COMMITTEE

1. Additional studies using narasin labelled with ^{14}C at several specific sites of the molecule confirmed that hydroxylation is the primary mode of narasin metabolism in chicken and that, among the six major metabolites isolated from chicken excreta, four are dihydroxy- and two trihydroxynarasins. Microbiological assays showed that these metabolites are 20 times less active than narasin on Bacillus subtilis. Individual biochemical testing of four of them (three dihydroxy- and one trihydroxynarasin) on rat liver cell mitochondria showed that they are much less effective than narasin in producing ionophorous effects.

(*) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 41

2. Additional studies on tissue residues were carried out in chickens fed 100 mg ^{14}C-narasin/kg feedingstuff for five days. All residue levels decreased by more than 50% during the first day withdrawal. After three days withdrawal, the total radioactive residues, expressed as narasin, were of the order of 0.1 mg/kg in liver, 0.03 mg/kg in kidney and skin and 0.01 mg/kg in fat and muscle. These levels had slightly decreased after five days withdrawal.

   A chromatographic separation completed with a bioautographic assay showed that residues in liver, kidney and muscle contained no unchanged narasin (limit of detection : 0.005 mg/kg) and that the small amounts of unchanged narasin in fat and skin had disappeared after two days withdrawal.

3. On the basis of these data, the Committee is of the opinion that the use of narasin can be admitted without risks in feedingstuffs for chickens at the levels provisionally authorized (60 - 80 mg/kg) and with a withdrawal period of at least five days before slaughter.

REFERENCES

Dossiers Lilly Research Centre Ltd. (1983)

THIRD REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF HALOFUGINONE IN FEEDINGSTUFFS FOR POULTRY

Opinion expressed 8 February 1984

TERMS OF REFERENCE (November 1977 and October 1981)

In reply to questions put by the Commission on the safety of use of halofuginone in feedingstuffs for chickens and turkeys, the Committee, in its reports of 25 April 1979 (*) and 17 November 1982 (**), considered that the proposed uses could be admitted provisionally with a withdrawal period of at least seven days before slaughter and that further information was necessary to issue a final opinion.

As the additional studies requested on the biodegradation of the product in the environment had been carried out, and assessed by the Committee in 1982 (**), alone some toxicological aspects of halofuginone needed to be clarified. As new experimental data on this topic are now available, the Committee expressed the following opinion.

OPINION OF THE COMMITTEE

The Committee has reviewed the results of four additional mutagenicity tests requested to clarify whether or not halofuginone had any genotoxic potential.

(*) Reports of the Scientific Committee for Animal Nutrition, second series (1980), No EUR 6918, p. 11

(**) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 94

The in vitro point mutation test in cultured mouse lymphoma cells strain L 51784 Y was negative both in the presence and absence of S 9 mix for metabolic activation. The in vivo bone marrow cytogenetics test in the rat showed no clastogenic effects in this species. An in vitro test for DNA repair synthesis in HeLa 53 epithelioid cells, using radiolabelled thymidine incorporation and autoradiography, gave erratic results which were not repeatable. Because of a vague indication that halofuginone might induce some DNA repair synthesis directly in the absence of S 9 mix, a test for covalent binding of halofuginone to the hepatic DNA of rats was carried out. Practically no covalent binding was detected.

In the light of these findings, the Committee considered that halofuginone had no genotoxic activity nor any mutagenic potential. This compound can be admitted without risks in feedingstuffs for chickens and turkeys at the levels provisionally authorized (2-3 mg/kg) and with a withdrawal period of at least five days before slaughter.

REFERENCES

HRC Reports PSL 563/83976, 564/82723, 64/83730

IBMC Report 0333-83

SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF SALINOMYCIN IN FEEDINGSTUFFS FOR CHICKENS

Opinion expressed 4 April 1984

TERMS OF REFERENCE (November 1980)

In reply to questions put by the Commission on the safety of use of salinomycin in feedingstuffs for chickens, the Committee expressed a favourable opinion on 14 April 1982 (*) on the provisional use of the additive in feedingstuffs for chickens, piglets, pigs and fattening cattle, subject to a withdrawal period of five days before slaughter. It was agreed that the product should be reassessed when additional data on the nature of tissue metabolites in the various species were available.

In the light of the information that has since been received on the use of the additive for chickens it is possible to interpret the original data more accurately. Accordingly, the Committee's opinion is now as follows.

OPINION OF THE COMMITTEE

1. The metabolism of salinomycin has been studied in the chicken, using a molecule labelled with ^{14}C at three specific sites, including one corresponding to the carboxylic function. After administration of single or multiple oral doses, most (up to 97%) of the radioactivity was recovered in the excreta within 72 hours. Almost all the activity was detected in the intestinal and caecal contents, showing that excretion via the urine is of limited significance.

(*) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 36

Measurable quantities of radioactivity (14% of the ingested dose) were excreted over a 72 hours period in the bile, indicating that salinomycin is partially absorbed. However, the very low levels measured in the blood and their limited persistence therein (1 hour) indicated that the salinomycin that is absorbed returns mainly to the intestine via the bile after metabolism in the liver. A very low excretion of $^{14}CO_2$ was observed.

Analysis of the excreted products showed the presence of a very small quantity of the unchanged antibiotic (< 1%) and a number of metabolites, of which the three main ones have been identified. One is a di-hydroxylated derivative and two are tri-hydroxylated, representing 11% and 28% respectively of the excreted radioactivity. Chromatographic analyses showed the presence of the same metabolites in the bile. It can be established from these data that salinomycin once absorbed is metabolized and that the metabolites are eliminated mainly via the bile and subsequently via the faeces. There was no qualitative or quantitative difference between males and females. Metabolites recovered from the excreta have a much lower antibiotic activity and acute toxicity (mouse) than salinomycin itself.

2. Following administration of salinomycin at the level of 66 g/ton of feedingstuff for 10 days and after a withdrawal period of 24 hours no antibiotic activity was detected in the chicken tissues (microbiological limit of detection, expressed as salinomycin : 0.01 mg/kg).

In another experiment covering 28 days of administration at dose-levels between 80 and 140 g/ton of feedingstuff, an antibiotic activity was measured after 17 hours withdrawal of salinomycin in the liver and kidneys (0.05 mg/kg, expressed as salinomycin) and subcutaneous fat (0.05 - 1 mg/kg, expressed as salinomycin). After 72 hours withdrawal, there was no more activity except in the liver of one animal where traces were detected. Under the same experimental conditions, the use of ^{14}C-salinomycin showed measurable residual

radioactivity in the liver, kidneys, muscle and adipose tissue 120 hours after cessation of administration. In further investigations salinomycin was identified in the adipose tissue and the liver after a withdrawal period of 17 hours, together with labelled triglycerides. After longer withdrawal periods (72 and 120 h), the residual radioactivity in adipose tissue and skin was mostly present in the lipids and resulted from the incorporation of labelled carbon fragments arising from the salinomycin molecule. It is likely that the insoluble radioactive fraction simultaneously present in the liver and kidneys has a similar origin.

3. On the basis of the available data, the Committee is of the opinion that salinomycin can be used without risks in feedingstuffs for chickens at the level provisionally authorized (50 - 70 mg/kg) and with a withdrawal period of not less than five days before slaughter.

REFERENCES

Dossiers Hoechst A.G. (1983)

SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF OLAQUINDOX IN FEEDINGSTUFFS FOR PIGS

Opinion expressed 3 May 1984

TERMS OF REFERENCE (July 1978)

In reply to questions put by the Commission on the safety of use of olaquindox in feedingstuffs for pigs, the Committee, in its report of 8 July 1981 (*), considered that the proposed use could be admitted provisionally and that a reassessment would be necessary once additional data on mutagenicity become available. The Committee noted that the mutagenicity studies then available appreared to be insufficient and requested further extensive studies using a battery of tests covering not only bacterial test systems but also those investigating other genetic endpoints in relation to chromosomal and DNA changes.

As the studies requested have been carried out and are now available, the Committee expressed the following opinion.

OPINION OF THE COMMITTEE

1. The Committee reviewed a total of 15 mutagenicity tests covering the following genetic endpoints : point mutations in prokaryotes and eukaryotes *in vitro* and *in vivo*, cytogenetic changes *in vitro* and *in vivo*, the latter by several routes of administration, and reaction with DNA. Practically all *in vitro* and *in vivo* tests were positive but only at near toxic doses. Covalent binding to DNA and those tests in mice, where the dosage used was low, were negative. The mouse appears to be comparatively insensitive to olaquindox yet most of the mutagenicity tests were performed in this species.

(*) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 1

However, a test for clastogenic potential in the Chinese hamster, a sensitive species, was positive. These findings show that olaquindox is genotoxic. It has no carcinogenic potential as determined in long-term studies.

2. Possible risks from dust inhalation were also studied. Feedingstuffs containing 50 mg olaquindox/kg feed were tested both in meal and pellet form under normal feeding conditions, the process of preparing the feed from premix containing olaquindox not having been examined for dust development. Feeding proceeded by distributing either 2 batches of meal or 1 batch of pellets to 5 pens over a period of 3 minutes. Air was sampled throughout the whole feeding period at the height and in the neighbourhood of the operator using an Andersen-Mark II-Cascade impactor. No olaquindox was detected on the filters or in the 24-hour urine sample of the operator. These findings confirm the opinion of the Committee stated earlier, that the risk to health incurred by farm workers is negligible under practical conditions of distribution of animal feed containing olaquindox.

3. The Committee recalls that residues resulting from the use of olaquindox in pig feed are below the detection limits after 48 hours withdrawal (limit of detection 0.1 mg/kg).

4. In the light of these data, the Committee is of the opinion that olaquindox fulfilling the specifications of the preparation investigated can be used without risks in feedingstuffs for pigs up to four months at the levels provisionally authorized (15-50 mg/kg of complete feedingstuffs; 50-100 mg/kg of milk replacers) and with a withdrawal period of at least four weeks before slaughter.

REFERENCES

Supplementary Dossiers Bayer A.G. (1983, 1984)

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF PANCOXIN (*) AND PANCOXIN PLUS (*) IN FEEDINGSTUFFS FOR POULTRY

Opinion expressed 3 May 1984

TERMS OF REFERENCE (April 1981)

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

1. Does the use of the coccidiostats Pancoxin (*) and Pancoxin Plus (*) in feedingstuffs for chickens and turkeys, under the conditions provisionally authorized (see Background), result in the presence of residues in animal products ? If so, what is the nature and the amount of these residues ? Could these residues be harmful to the consumer ?

2. Could the use of these additives affect the development of resistance in bacteria ?

3. Could the excreted products derived from these additives be prejudicial to the environment ? If so, what is the nature of the risks ?

4. In the light of the answers to the above questions, are the conditions of use of these additives acceptable ?

BACKGROUND

In accordance with the provisions of Council Directive 70/524/EEC, of 23 November 1970, concerning Additives in feedingstuffs (1), as last amended by the thirty seventh Commission Directive of 9 April 1981 (2),

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(*) Registered trade name
(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 131, 18.05.1981, p. 1

Member States are authorized by way of derogation to use Pancoxin (*) and Pancoxin Plus (*) up to the 30 November 1981 under the following conditions set out in Annex II, Section B, of the Directive :

Additive	Species of animal	Min. content: ppm (mg/kg) of complete feedingstuffs	Max. content: ppm (mg/kg) of complete feedingstuffs	Other provisions
Pancoxin (*) : mixture of 18 parts amprolium (a), 10.8 parts sulphaquinoxaline (b) and 0.9 parts ethopabate (c)	Chickens for fattening, turkeys	-	(a): 100 (b): 60 (c): 5	Use prohibited at least seven days before slaughter
Pancoxin Plus (*) : mixture of 20 parts amprolium (a), 12 parts sulphaquinoxaline (b), 1 part ethopabate (c) and 1 part pyrimethamine (d)	Chickens for fattening	-	(a): 100 (b): 60 (c): 5 (d): 5	Use prohibited at least seven days before slaughter

OPINION OF THE COMMITTEE

The Committee noted that the studies on metabolism, residues and excreted products from Pancoxin (*) and Pancoxin Plus (*), available in 1981, were insufficient and requested that further extensive studies be carried out. As supplementary dossiers were now available, the Committee expressed the following opinion.

1. The individual constituents (amprolium, sulphaquinoxaline, ethopabate and pyrimethamine) of Pancoxin (*) and Pancoxin Plus (*) have been examined toxicologically but not all substances have been studied to

the same extent. No toxicity or residue data are available on chickens and turkeys fed on Pancoxin (*). Metabolism studies are not available for pyrimethamine in chickens and for amprolium and ethopabate in turkeys. Residue data are available only for chickens fed on amprolium, sulphaquinoxaline and ethopabate or Pancoxin Plus (*) and for turkeys fed on sulphaquinoxaline. No information was supplied on the nature of the residues for any of the constituent substances. Therefore, the Committee considered that the available information summarised below is still insufficient to reply fully to question No 1 put by the Commission. It appeared however that residue data of sulphaquinoxaline point to the need for a withdrawal period of at least 7 days in chickens and at least 14 days in turkeys.

1.1. Amprolium

Metabolism was studied in chicken using orally administered ^{14}C radiolabelled material. Over 90% of radioactivity was excreted in 48 hours, about 75% being unchanged amprolium.

Residues in chicken fed 125-250 mg/kg in the feed for up to 8 weeks were highest in kidneys but low in liver and muscle. After 3 weeks feeding, residues ranged from 1-3 mg/kg in kidneys, 0.7-1.7 mg/kg in the liver to 0.2 mg/kg in muscle. After 4 days withdrawal, residues were less than 0.01 mg/kg in all tissues (limit of detection 0.01-0.02 mg/kg).

Toxicity studies were performed in mice, rats and dogs. The oral LD_{50} in mouse and rat was 4 mg/kg b.w. Ninety-day studies in rats and dogs produced weight loss, diarrhoea and increased mortality with the no-effect-level being 200 and 400 mg/kg b.w. A two year study in the rat was inadequate to determine a no-effect-level but showed no carcinogenic effect. High doses reduced growth and survival. The two year dog study suggests a no-effect-level of 100 mg/kg b.w. No reproduction or teratology studies were available. Mutagenicity tests in prokaryotic systems were negative.

1.2. Sulphaquinoxaline

Studies of the metabolism in chickens and turkeys showed rapid absorption with dose-dependent plasma levels persisting for up to 12 hours. Chickens acetylate the compound to a small degree. Most species form the insoluble 3-hydroxy derivative which is deposited as renal crystals. There is little information on metabolites. Sulphaquinoxaline diffuses into eggs in proportion to the plasma levels.

Broilers treated for 2 weeks with a subsequent 7-day withdrawal period had residues in the skin of 0.14 mg/kg and in liver, kidneys, muscle and fat below 0.1 mg/kg (sensitivity of method 0.1 mg/kg). Residues in cockerels treated for 2 weeks followed by a 7-day withdrawal period were 0.28 mg/kg in kidneys, 0.1 mg/kg in the liver, 0.11 mg/kg in the skin and less than 0.1 mg/kg in muscle and fat. Residues in the liver, kidneys, muscle and skin of turkeys were below 0.15 mg/kg after 10-14 days withdrawal periods. Broilers treated for 43 days with Pancoxin Plus (*) (60 mg/kg feed sulphaquinoxaline) had no detectable residues after a 5-day withdrawal period (sensitivity of method not stated).

The toxicity has been studied in several species. The oral LD_{50} for mice is 15 g/kg b.w., for rats 1 g/kg b.w. The main toxic effects are interference with blood clotting mechanism, tubular nephropathy with renal crystal deposition and focal hepatic necrosis. A 90-day study in rats showed enlargement of the thyroid with a no-adverse effect level of 2 mg/kg b.w., but longer studies at high levels produced tubular nephropathy, enlargement of the thyroid and testicular atrophy. A 90-day study in beagle dogs gave similar results with a no-effect-level of less than 2 mg/kg b.w. A large number of short-term studies in chicken and turkeys produced haemopoietic effects at high doses but 120-500 mg/kg b.w. were tolerated over several months. A long-term study, multigeneration reproduction study and

teratology study in rats showed no abnormal effects at dose levels of 0.38-2.5 mg/kg b.w. The mutagenicity studies in prokaryotic systems were negative.

1.3. Ethopabate

The metabolism was studied in rats, dogs and chicken. Single oral doses were almost entirely excreted within 24 hours in the urine of all species, almost all as unchanged etho- pabate. Chicken excreted 87-100% of oral single doses and 0.07-11% as CO_2, determined by the use of radiolabelled ma- terial. The proportion of radioactivity exhaled depended on the position of radiolabel. No metabolites or ethopabate were dete- cted in the tissues. The metabolite 4-acetylamino-2-ethoxyben- zoic acid was identified in the urine of chicken.

Residue studies in chicken with repeated doses showed the highest levels when the aromatic ring was labelled. Levels in the kidneys were 1.3 mg/kg, in the liver 0.9 mg/kg and in muscle 0.2 mg/kg. After 5 days withdrawal residues were 0.05 mg/kg in all tissues (sensitivity of method 0.05 mg/kg). If the ethoxy group was labelled, residues ranged from 0.05-0.3 mg/kg depending on dose level and no radioactivity was detectable after 8 hours in any tissue. If the carboxy group was labelled, residues were 0.3 mg/kg in muscle, 1 mg/kg in kidneys and 0.6 mg/kg in the liver, reducing to 0.05 mg/kg or less after 5 days withdrawal.

Toxicity studies include oral LD_{50} in mouse and rat (about 14 g/kg b.w.) and short-term studies in rat, dog and chicken. Rat and dog studies with doses ranging from 10 mg/kg to 5 g/kg b.w. pro- duced mainly hepatotoxicity, the no-effect-level for the dog being 10 mg/kg b.w. and for the rat 100 mg/kg b.w. Feed intake, egg production and hatchability in chicken were adversely affec- ted by 500 mg/kg in the feed.

Long-term studies in rats and dogs showed only hepatotoxic effects but no carcinogenic potential. No reproduction or teratology studies were available. Mutagenicity studies in prokaryotes and Saccharomyces were negative.

1.4. Pyrimethamine

Metabolic studies in mice, rabbits and monkeys show that 50-60% of single oral doses are excreted in the faeces within 24 hours. Man excretes pyrimethamine slowly, about 12% of the dose appearing in the urine within 5 days but excretion is still detectable in the urine for 11 days and in the blood for 7 days.

The toxicity has been studied in several species. The oral LD_{50} for mice is 90 mg/kg b.w. Short-term studies extending over 42 to 90 days in rats, dogs and monkeys showed the main toxic effect to be bone marrow depression together with other species specific adverse effects on growth, testes, kidney and the gastrointestinal tract. The no-adverse-effect level varied from 1.25 mg/kg b.w. in monkeys and dogs to 2.5 mg/kg b.w. in rats. Chickens showed similar bone marrow disturbances when treated for 56 days with 20 mg/kg b.w. Carcinogenicity studies in mice and rats revealed no tumorigenic activity. A multigeneration-reproduction study and teratology studies did not suggest any adverse reproductive effects. Mutagenicity tests in prokaryots bacteria were negative, but genotoxic activity was noted in Drosophila and clastogenic activity in bone marrow of mice.

2. Of the substances contained in Pancoxin (*) and Pancoxin Plus (*), sulphaquinoxaline and pyrimethamine have antibacterial properties. Their activity is less than that of sulphamethazol or trimethoprim, however. In Pancoxin Plus (*) the two substances show none of the synergistic effects which have been described in the case of sulphame- thazol and trimethoprim (Guinée 1974, Walter and Heilmeyer 1975).

The effects of sulthaquinoxaline on the percentage of sulphonamide-resistant E. coli in the intestinal flora of chickens were studied in vitro and in vivo (Guinée 1974, Guinée and Kruyt 1975). No significant difference was observed between treated and untreated animals. However, these experiments did not conclusively demonstrate that the use of Pancoxin (*) or Pancoxin Plus (*) in animal nutrition has no effect on the persistance of E. coli multiresistant to drugs since the percentage of these bacteria in the intestinal flora of the experimental animals was very high.

It was found, however, that the resistance to sulphonamides, being located on plasmids, would only exceptionally take the form of mono-resistance in E. coli (Renault 1974, Renault and Decourneau 1975). Sulphonamide-resistant E. coli strains show in most cases anything up to a five-fold resistance against antibiotics (Renault and Decourneau 1975, Gedek 1980, 1981, 1982, Siebert 1982, Pohl 1983). The hypothesis that, due to the presence of a quinoxaline cyclic ring, sulphaquinoxaline might have antibacterial properties which are different from those of other sulphonamides has not been demonstrated. No data are available on the question of whether a loss of R-factors may, as in the case of aromatic N-dioxides, result from the administration of sulphaquinoxaline. The available data thus do not allow a reply to question No 2 put by the Commission.

3. Experimental data on the excretion of the individual constituents of Pancoxin (*) and Pancoxin Plus (*) are available for chickens only. Amprolium and ethopabate are eliminated unmetabolized in droppings; sulphaquinoxaline is eliminated for the most part unmetabolized and for a small part as an acetyl derivative while pyrimethamine is excreted as a labile acid conjugate. Amprolium is highly hydrosoluble; sulphaquinoxaline, ethopabate and pyrimethamine are barely soluble. The four compounds showed little toxicity for algae, crustacea and fish, as indicated by the data below :

Chlorella : EC_{50} (mg/l, 2 days) : amprolium : 160, pyrimethamine : 20

Daphnia : EC_{50} (mg/l, 2 days) : amprolium : 230, ethopabate : 170, sulphaquinoxaline : | 7.5, pyrimethamine : 4.8
Lebistes : EC_{50} (mg/l, 2 dyas) : amprolium : 270. LC_{50} (mg/l, 2 days) : ethopabate : 105, sulphaquinoxaline : | 7.5, pyrimethamine : 7.5
Salmo gairdneri : LC_{50} (mg/l, 2 days) : amprolium : 1550, ethopabate : 23, sulphaquinoxaline : | 7.5, pyrimethamine : 5.9.

The phytotoxic potential of Pancoxin Plus (*) has been tested on eight plant species at concentrations between 1 and 1.000 mg/kg soil. No phytotoxicity was observed for lettuce, beans, peas and sunflower. A 50% reduction in plant growth was observed at high concentrations (between 285 and 1067 mg/kg soil) on oats, rape, tomatoes and maize.

In vitro studies showed that Pancoxin (*) as well as Pancoxin Plus (*) do not substantially affect methanogenesis and reduce nitrification only at concentrations distinctly in excess of those that can be attained in soil after spreading of slurry.

The results currently available, although incomplete, suggest that these substances do not constitute a significant environmental risk.

4. In view of the foregoing, the Committee considers that it is unable to issue an opinion on the use of Pancoxin (*) and Pancoxin Plus (*) in feedingstuffs for chickens and turkeys before the following additional data are available :

a) reproduction and teratology studies on amprolium and ethopabate;
b) in vivo mutagenic studies (chromosomal effects) of amprolium, sulphaquinoxaline and ethopabate;
c) metabolism and tissue residues of pyrimethamine in chicken;
d) metabolism and tissue residues of amprolium and ethopabate in turkey;

e) data showing that the metabolism and residues of individual components are not affected when the mixture Pancoxin (*) or Pancoxin Plus (*) is used;

f) effects of Pancoxin (*) and Pancoxin Plus (*) on the persistance of E. coli multiresistant to drugs in the alimentary tract of treated animals;

g) kinetics and degradation of amprolium, sulphaquinoxaline, ethopabate and pyrimethamine in soil and water.

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SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF LERBEK (*) IN FEEDINGSTUFFS FOR TURKEYS

Opinion expressed 11 July 1984

TERMS OF REFERENCE (July 1978 expanded in October 1981)

In reply to questions put by the Commission on the use of Lerbek (*) (premix containing 100 parts of meticlorpindol and 8.35 parts of methylbenzoquate) in feedingstuffs for turkeys, the Committee, in its report of 17 November 1981 (**), delayed expressing its opinion until data on metabolism of Lerbek (*) in turkeys, its residues and excreted products became available. Since the appropriate studies have now been carried out the Committee expressed the following opinion.

OPINION OF THE COMMITTEE

1. Residues of meticlorpindol (MCP) in tissues and excreta have been studied in two groups of turkeys. In the first one, the birds were given a daily dietary administration of MCP (100 mg/kg feed) for 14 days followed by a single oral dose of ^{14}C-MCP (6.5 mg/kg feed). The MCP (3,5-dichloro-2,6-dimethyl-4-pyridinol) was ring labelled in positions 2 and 6. The birds were then slaughtered after 24 h, 48 h or 72 h. To the second group, Lerbek (*) was given for 14 days followed by a single oral dose of ^{14}C-MCP (6.5 mg/kg feed) and unlabelled methylbenzoquate (MBQ) (0.54 mg/kg feed). In both treatment groups a mean value of about 90% of the radioactivity administered was collected in the combined excreta during a 72 hour period following dosage with the labelled MCP. Approximately half was present as unchanged MCP, the remainder being non identified.

(*) Registered trade name
(**) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 87

The level of radioactivity in tissues, expressed as ug equivalents ^{14}C- MCP/g, ranged, after 72 h, from 0.3 to 1.1 in liver and kidneys, and 0.1 to 0.4 in muscle. Unchanged MCP accounted for 59-82% of the radioactivity. The similarity of the results obtained with both treatments showed that MCP metabolism is not changed in presence of MBQ.

In turkeys given Lerbek (*) at the proposed level (110 mg/kg feed) for a period of 12 weeks and then sacrified at various times thereafter, no residues of MBQ were detected (limit of detection : 0.02 mg/kg) in muscle, liver and kidney after a 24 hour-withdrawal period and no residues of MCP were detected (limit of detection : 0.05 mg/kg) after a 7 day-withdrawal period.

From the foregoing it appears that the use of Lerbek (*) in feedingstuffs for turkeys gives comparable residues in tissues to those resulting from its use in chickens.

2. Also from the foregoing and the relevant data given in the report on the use of lerbek (*) in feedingstuffs for chickens (**), it appears that this product can be used in feedingstuffs for turkeys without risks for the consumer or the environment.

3. In the light of these findings, the Committee is of the opinion that the conditions proposed for the use of Lerbek (*) in feedingstuffs for turkeys (110 mg/kg complete feedingstuffs and a withdrawal period of at least 5 days before slaughter) are acceptable.

REFERENCES

Dossiers Dow Chemical and HRC (1984)

REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF ZINC BACITRACIN IN FEEDINGSTUFFS FOR POULTRY, PIGS AND RABBITS

Opinion expressed 30 January 1985

TERMS OF REFERENCE (September 1980)

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

1. Does the addition of zinc bacitracin to complete feedingstuffs in dose levels higher than 20 mg/kg produce a significant increase of nutritional effects, free of prophylactic or therapeutic effects, in the case of poultry (excluding turkeys, ducks, geese, laying hens and pigeons) over the age of four weeks, swine over the age of four months, and rabbits ?
   If so, what is the dose/response ratio ?

2. Is the administration of complete feedingstuffs with a maximum zinc bacitracin content of 100 mg/kg justified from a nutritional point of view
   - in the feeding of poultry (excluding turkeys, ducks, geese, laying hens and pigeons) up to the age of 16 weeks,
   - in the feeding of swine up to the age of six months,
   - in the feeding of rabbits ?

3. Does the use of zinc bacitracin in feedingstuffs for poultry, pigs and rabbits, under the conditions of use being proposed (cf. Background), result in the presence of residues in animal products ?
   If so, what is the nature and the amount of these residues ? Could they be harmful to consumers ?

4. Could these conditions of use lead to the development of resistance in bacteria ?

5. Could they be harmful to the environment ?
   If so, what is the nature of the risks ?

6. In the light of the answers to the above questions, are the conditions of use being proposed acceptable ?

BACKGROUND

In accordance with Council Directive 70/524/EEC of 23 November 1970 concerning additives in feedingstuffs (1), as last amended by the 34th Commission Directive of 4 September 1980 (2), the use of zinc bacitracin is authorized throughout the Community, subject to the conditions of use set out as follows in Part A of Annex I to the Directive :

Species of animal	Maximum age	Minimum content ppm (mg/kg) of complete feedingstuff	Maximum content ppm (mg/kg) of complete feedingstuff
Turkeys	4 weeks,	5	50
	from 4 to 26 weeks	5	20
Poultry, excluding turkeys, ducks, geese, laying hens and pigeons	4 weeks,	5	50
	from 4 to 16 weeks	5	20
Laying hens	-	15	100
Calves, lambs and kids	16 weeks,	5	50
	from 16 weeks to 6 months,	5	20
	6 months	5	80 (*)
Piglets	4 months	5	50
	6 months	5	80 (*)
Swine	from 4 to 6 months	5	20
Animals bred for fur	-	5	20

(*) Milk replacers

(1) OJ No L 270, 14.12.1970, p. 1
(2) OJ No L 251, 24.09.1980, p. 17

The proposed amendments to these conditions of use are given in the table below :

Species of animal	Maximum age	Minimum content ppm (mg/kg) of complete feedingstuff	Maximum content ppm (mg/kg) of complete feedingstuff
Turkeys : no change			
Poultry, excluding turkeys, ducks, geese, laying hens and pigeons	16 weeks	5	100
Laying hens : no change			
Calves, lambs, kids : no change			
Swine, piglets	6 months	5	100
Rabbits	-	50	100

In the opinion delivered on 21 February 1978 (3), the Scientific Committee for Animal Nutrition reserved its position regarding any increase in the dose levels authorized for zinc bacitracin, given the lack of supporting data. As further relevant material is now available, the Commission considers that the matter should be re-examined.

OPINION OF THE COMMITTEE

The dossiers made available to the Committee were those on zinc bacitracin produced by A/S Apothekernes Laboratorium for Special-praeparater.

(3) Reports of the Scientific Committee for Animal Nutrition, First Series (1979). Office for Official Publications of the European Communities, Luxembourg. Catalogue No CB-28-79-277-FR-C.

Considering that the data available up to 1982 did not enable a satisfactory assessment to be made of the effects of an increase in the dose levels of this antibiotic, the Committee requested that additional studies be carried out on the efficacy of the product, the matabolism and residues, mutagenicity, bacterial resistance and the effects on the environment. The results of these studies were submitted during 1984. The opinion delivered hereinafter is none the less restricted to broilers and pigs, the only target species to have been the subject of appropriate studies under the newly proposed conditions of use.

1. and 2. The nutritional effects of zinc bacitracin were established on the basis of 839 tests involving 178 000 broilers and 207 tests ivolving 6 400 pigs. The parameters considered were the increase in liveweight gain and the improvement in the feed conversion ratio among the groups given feed containing zinc bacitracin as compared with the control groups. The zinc bacitracin content of the additive-containing feeds varied between 3 and 275 mg/kg for the fowl and between 3 and 250 mg/kg for the pigs.

   Processing the results by multiple regression analysis enabled a statistically significant dose/response ratio to be worked out for each parameter and allowed the optimum concentrations of the additive to be assessed for broilers and pigs (Morris 1983). On account of the high number of variables associated with the trials (breed, ration composition, breeding conditions, etc.), the parameters included in the multiple regression equation enable the variance to be explained in part only (21 to 32%).

   For broilers, the optimum zinc bacitracin concentration is 110 mg/kg of feed for the increase in liveweight gain and 130 mg/kg of feed for the improvement in the feed conversion ratio. For pigs, the optimum concentration is 106 mg/kg of feed for the increase in liveweight gain and 125 mg/kg of feed for the improvement in the feed conversion ratio.

For both species, the favourable effects of the additive up to optimum concentration show a gradual reduction with the increase in concentration.

Although the yield is not proportional to the dosage used, the increase in the concentration of zinc bacitracin in feedingstuffs for broilers and pigs up to 100 mg/kg feedingstuff is justified by statistically significant nutritional effects. Since these studies were performed on healthy animals, the likelihood of prophylactic or specific therapeutic effects can be ruled out.

3. Bacitracin is a polypeptide antibiotic produced by Bacillus licheniformis; its main components are the dodecapeptides, bacitracins A, B_1, B_2 and F_1. The acute toxicity of bacitracin has been determined in the mouse, rat, rabbit and dog by various routes of administration. The subacute toxicity has also been tested in the mouse, rat, rabbit, dog and monkey by various routes, including a subchronic oral feeding test in rats extending over 90 to 365 days. No toxic effects were noted. Furthermore, bacitracin did not irritate skin or eyes.

Zinc bacitracin was tested for mutagenicity in the Salmonella reverse mutation test, in a mouse lymphoma gene mutation test, an in vitro test for chromosomal aberrations in human peripheral lymphocytes and in rat bone marrow cells. No mutagenic activity was detected. DNA metabolism in spleen cells in vitro showed also no adverse effects. Thus bacitracin lacks mutagenic activity and there is therefore no reason to suspect genotoxic carcinogenic activity.

Bacitracin is appreciably resistant to hydrolysis by mammalian proteases and peptidases due to its cycloheptapeptide substructure, its thiazoline ring in the N-terminal L-isoleucine-L-cysteine moiety and to the presence of four D-amino acids (D-glutamine, AA 4, D-ornithine, AA 7, D-phenylalanine, AA 9, D-aspartic acid, AA 11). Only some 4% of ingested bacitracin is broken down to di- and tripeptides and free amino acids.

Zinc bacitracin metabolism has been studied in rats, chickens and piglets using single oral doses of the antibiotic labelled with ^{14}C-L-isoleucine in AA positions 1, 5 and 8 in the molecule. The doses administered were 80 mg/kg live weight for the rat, 20 mg/kg live weight for the chicken and 7 mg/kg live weight for the piglet; with the piglets dosing with the labelled product was preceded by the administration of a ratio containing 117 mg zinc bacitracin/kg feed for 18 days. Of the administered dosages, 102% was recovered in rat faeces and 96% in piglet faeces; with the chicken 95% of the administered dose was recovered in the combined excreta (faeces and urine). Analysis by TLC/autoradiography of the rat and piglet faeces and the chicken excreta showed the presence of oxidised derivatives (i.e. bacitracins F) of the bacitracins A, B_1 and B_2, of desamidobacitracins and di- and tripeptides in addition to the original bacitracin supplied in the feed. Rat and piglet urine contained respectively 3.2 and 3.6% of the administered dose while the comparable values for bile were 0.5% and 0.03% respectively. No bacitracins or their near derivatives were detected in the bile or urine, nor was there any ^{14}C-labelled free isoleucine.

Traces of radioactivity were found in some samples of liver and kidney from the chick (limit of determination 0.027 mg/kg fresh weight) and in liver, kidney, lung and heart of the piglet (limit of determination 0.0038 mg/kg fresh weight). The negligible quantities of these tissue residues, which persisted for some 5-7 days in some animals after withdrawal of the antibiotic from the feed, were not identified. However, extracts of the liver failed to show any microbiological activity. Tissues from chicken and piglets kept on feeds containing 100 mg or more zinc bacitracin for periods up to one month or more contained no bacitracin detectable by microbiological assay (limit of determination 0.054-0.26 mg/kg). Therefore, no harm to the consumer arises.

4. The administration of zinc bacitracin in various concentrations up to 100 mg/kg feedingstuff to pigs and broilers only slightly altered the number of bacteria in the intestinal flora as far as the main species

were concerned, i.e. lactobacilli, enterococci, E. coli and staphylococci (Gedek, 1983; Walton, 1983).

The slight increase in the MIC values observed mainly in the enterocci vis-à-vis bacitracin used as a feed additive was not accompanied by reduced sensitivity to other antibiotics used therapeutically (inter alia ß-lactam antibiotics, tetracyclines and chloramphenicol). Furthermore, after administration of zinc bacitracin to pigs and chickens for several weeks, a lowering of the resistance of enterococci and E. coli in the intestinal flora of these animals to several drugs (antibiotics, sulphonamides, nitrofurans, etc.) used in human and veterinary medicine was noted. The concentration of 100 mg/kg of feed led to a greater reduction that did 20 mg/kg (Walton 1978, 1983; Walton and Laerdal 1980; Siebert, 1982).

5. After administration of zinc bacitracin, the products excreted by pig and chicken were made up of bacitracin A, B and F, desamidobacitracins, breakdown products and non-extractable components bound to faeces constituents. Their antibiotic activity fell sharply in excreta and soils. The half-life of zinc bacitracin mixed with the excreta of chicken is two to seven days depending on the temperature. This rapid loss of antibacterial activity due to oxidative deamination means that any effect on the nitrifying bacteria in the soil may be ruled out. Nitrogen-fixing bacteria (Rhizobium trifolii) are not sensitive to zinc bacitracin, even in high concentrations. Under experimental conditions, bacitracin inhibits the formation of methane in sludges even in a 3 mg/kg concentration (Hilpert et al., 1983). On account of its rapid inactivation in excreta however, it does not seem that in practice, zinc bacitracin could affect the production of biogas.

Bacitracin does not present any risk to fish, shellfish or algae. No adverse effect was observed on trout (Salmo gairdneri) fed for 110 days on feed containing 57.5 and 104 mg zinc bacitracin/kg respectively. The CL_{50} after 96 hours for this species is 74 mg/l.

For Daphnia magna, the minimum concentration without any effect is 3.2 to 5.6 mg/l, and the CL_{50} after 48 hours is 34 mg/l. The growth of green algae (Chlorella ellipsoides) is not affected by zinc bacitracin at a concentration of 10 mg/l.

No inhibiting effect on the growth of plants was observed in plant species grown in soils mixed with manure from poultry fed with feed containing from 20 to 100 mg zinc bacitracin/kg or poultry manure mixed with 150 mg zinc bacitracin/kg.

6. On the basis of the foregoing information, the Committee is of the opinion that the use of zinc bacitracin levels of 5-100 mg/kg complete feedingstuffs for broilers up to the age of 16 weeks and pigs up to the age of six months is justified for the animal production and does not present any risk for human or animal health or for the environment.

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SECOND REPORT OF THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION ON THE USE OF NICARBAZIN IN FEEDINGSTUFFS FOR FATTENING CHICKEN

Opinion expressed 5 July 1985

In accordance with its opinion delivered on 14 April 1982 (*) and having received from the firm Merck, Sharp and Dohme further documentary information on nicarbazin, the Committee reassessed the use of the additive in feedingstuffs for fattening chicken. For the purposes of reassessment, all the data on nicarbazin supplied up to June 1985 were taken into consideration.

Further studies on residues in the tissues of chicken (liver, kidney, muscles, skin, fatty tissues) carried out in 1983 and using an improved polarographic method to determine the levels of DNC (limit of determination expressed in terms of nicarbazin : 0.1 mg/kg) have shown that the actual values are higher than those previously estimated by extrapolation from results obtained using a low-sensitivity polarographic method. No data have been made available as to the metabolism of nicarbazin in chicken.

Additional studies carried out in 1983 and 1985 on the environmental effects of nicarbazin have shown that nicarbazin is not phytotoxic and has no significant effects on aquatic life, methanogenesis or bacterial nitrification. No data have been made available on the biodegradability in dung and soil of excreted products derived from nicarbazin.

While taking account of the additional information on tissue residues, the Committee considers that, in the absence of data on the metabolism of nicarbazin and the qualitative composition of its residues, the withdrawal period for the supplemented feedingstuff before slaughter should be extended to 9 days, at least so that the residue levels estimated by determination of DNC do not exceed 0.2 mg/kg.

(*) Reports of the Scientific Committee for Animal Nutrition, fourth series (1984), No EUR 8769, p. 51

However, the Committee is unable to deliver a final opinion as to whether the use of nicarbazin is harmless until appropriate studies have been carried out on the metabolism of the product in chicken and the biodegradation of its excreted products in dung and soil.

REPORT ESTABLISHED JOINTLY BY THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION AND THE SCIENTIFIC COMMITTEE FOR FOOD ON THE USE IN ANIMAL NUTRITION OF PROTEIN PRODUCTS OBTAINED FROM CANDIDA YEASTS

Opinion expressed 3 May 1984

TERMS OF REFERENCE (January 1983)

The Scientific Committee for Animal Nutrition and the Scientific Committee for Food are requested to give their opinion on the following questions :

1. Do the products obtained from yeasts of the Candida variety and, in particular, from those cultivated on n-alkanes have a nutritional value for animals because they provide nitrogen or protein ?

2. Can the use in animal nutrition of products obtained from yeasts of the Candida variety and, in particular, from those cultivated on n-alkanes result in risks for human (consumer or user) or animal health, or be prejudicial to the environment ?

BACKGROUND

In accordance with the provisions of Council Directive 82/471/EEC of 30 June 1982 concerning certain products used in animal nutrition (0), Member States may, until such time as a Community decision has been taken, maintain authorizations granted within their territories before notification of the Directive concerning on the one hand products obtained from yeasts of the Candida variety and cultivated on n-alkanes and on the other hand products listed in Section 1.2.1. of the Annex to the Directive (yeasts cultivated on substrates of animal or vegetable origin) meeting requirements different from those laid down therein.

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(0) OJ No L 213, 21.07.1982, p. 8.

In accordance with the established procedure, the Commission consults the Scientific Committee for Animal Nutrition and the Scientific Committee for Food before producing a draft of the Community measures to be adopted for the compounds concerned.

OPINION OF THE COMMITTEES

The Committees draw the attention of the Commission to the fact that yeasts of the Candida variety are not a homogeneous group of micro-organisms in relation to their nutritional value and their pathogenicity (difference in virulence) for man and animals.

The Committees have made some general comments on the nutritional and pathogenicity aspects of Candida yeasts. In the absence of other relevant documentation they have evaluated the safety of only two specific products derived from two strains of Candida on which information has been provided, namely Toprina (*) and Liquipron (*).

Toprina (*) and Liquipron (*) are trade-marks for dried whole yeasts obtained by growing Candida lipolytica strain 246 and Candida strain ATCC 20275 IS respectively on n-alkanes containing culture media. The term Candida tropicalis is used in the text. However, there is no complete agreement on the actual classification of the Candida strain used as the source material for Liquipron (*).

1. Nutritional aspects

Candida yeasts may be broken down into groups according to the various substrates on which they can be grown, as indicated in the table below.

(*) Registered trade name

n-Alkanes	Methanol	Effluents and cellodextrins	Whey
C. tropicalis C. lipolytica C. utilis (b) C. maltosa (11)	C. boidinii (1)	C. pelliculosa (13) C. wickerhamii (a)	C. fabrianii (10) C. frigida (11) C. krusei (20)

(a) for the production of methanol

(b) also grown on sulphite solution (12), molasses and other sugar by-products

Extensive literature on the nutritional value of their proteins is available for C. lipolytica and C. tropicalis strains cultivated on n-alkanes. The crude protein content (N x 6.25) of products obtained from these micro-organisms varies from 60% to more than 70% on the basis of dry matter. Their protein efficiency (ratio between animal weight gain and quantity of proteins ingested) has been determined in various animal species (2, 3, 4, 5, 6, 8, 9, 19). In the rat, this efficiency is around 2.2-2.5 when the ration has been supplemented with 0.3% DL-methionine.

Except for low methionine levels, the amino-acid composition of C. lipolytica and C. tropicalis proteins is close to that of the egg. Lysine and threonine, limiting factors of the usual pig and poultry rations, are supplied in large measures by proteins obtained from Candida strains. At levels of 15% in the ration, these products cover 88% of chickens' and more than 100% of piglets' lysine requirements.

Studies on rats have shown that, at incorporation rates higher than 15% in the ration, the protein efficiency of products from C. lipolytica drops as the concentration increases in the same way as that of casein and whole egg which has been freeze-dried and heated at 60°C (7).

Provided nutritional balance is maintained, products from C. lipolytica and C. tropicalis strains can be used at levels up to 15%

in the rations of pigs, calves, lambs, poultry, pets, with the exception of the Dalmatian dog whose ration should not contain more than 3-4% of these yeasts (18). On the other hand, fish can be given high levels of them in their ration (14, 15, 16, 17). On account of their content in nitrogen compounds with high biological value, these products have a nutritional value comparable to or even higher than that of other sources of proteins commonly used in animal feed.

2. Risk assessment

2.1. Pathogenicity and hypersensitivity

2.1.1. General considerations on Candida strains

Viable Candida yeasts are so-called opportunistic pathogens, i.e. they may induce in predisposed hosts (human or animal organism) various forms of mycoses. Depending on exposure conditions, specific virulence of the strain and host factors, these mycoses may range from mild superficial infections to deep organ invasions (with predilection for kidney and brain) and generalized septicaemia which, if untreated, rapidly causes death (1, 6, 7). The skin, oral cavity and urogenital tract are more commonly affected in human beings, whereas the digestive tract is usually involved in young livestock (7, 8). Sometimes, a mycoallergy develops in addition to the underlying mycoses (7, 9). Both an overt and a latent infection during pregnancy may have serious consequences if viable cells of an opportunistic Candida strain are transmitted to the newborn at delivery.

Over the past ten years infections caused by Candida species have increased noticeably in number, severity and spectrum of causative species (14). This increase is reasonably attributed to the increase in predisposing factors which are generally a prerequisite for an organism being affected by a

candidosis. A severe form of candidosis may also occur in a healthy host when a high number of viable cells of the most virulent strains is administered. In humans, the predisposing factors include several metabolic disorders (e.g. diabetes), underlying bacterial or viral infections, treatment with broad- spectrum antibiotics, lowered resistance due to exposure to immunosuppressants (cytostatics or corticosteroids), congenital myelodeficiency and malignant disease (8, 14).

Candida-induced mycoses very often occur in animals in connection with the use of chemotherapeutics which, in warm-blooded animals, favour the development of yeasts by suppressing the normal intestinal flora (6). The use of medicated feedingstuffs may also result in an increased excretion of viable Candida yeast cells (5). Recently Candida yeasts have been shown to induce diseases also in freshwater fish (4).

Not all species of Candida are endowed with pathogenic properties for human or animal organisms (1, 6, 12, 14, 17, 16); this only happens when the optimal growth of the yeast occurs at the host body temperature and the yeast is able to withstand the various defence mechanisms that the host puts into play (among which phagocytosis is of utmost importance). The formation of mycelia or pseudomycelia is widely held to account for increased resistance of Candida to phagocytosis and for virulence in general (14), but other factors may contribute to the invasive properties of the micro-organism, particularly enzymes (mainly proteases) and cell glycoproteins (endotoxin-like substances) (7, 10, 16, 3).

A review of the current literature, covering both experimental and clinical reports, clearly shows that the following eight species of Candida are true pathogens (in this context "pathogenic" means "capable of inducing harmful effects in a

compromised host" with the understanding that the virulence will depend on the particular strain) : C. albicans; C. tropicalis; C. stellatoidea; C. glabrata; C. parapsilosis; C. pseudotropicalis; C. guilliermondii; C. krusei. According to Odds (14), all the traditional Koch's postulates, which still remain the orthodox criteria for judging a species to be regarded as pathogenic, are satisfied for these eight Candida species.

Moreover, the following Candida species have been occasionally isolated from human or animal tissues: C. claussenii; C. intermedia; C. brumptii; C. lipolytica; C. solani; C. ravantii; C. pulcherrima; C. ingens; C. lambica; C. macedoniensis and C. norvegensis. For these Candida species a strong aetiological association with a disease has not been documented but is, however, suspected. Lastly, C. viswanathii has been shown recently to be highly pathogenic in the mouse (2). C. ingens has been reported as a cause of septicaemia in surgical patients (15) and strains of C. utilis have been described which are virulent for the mouse and possess very active cytoplasmic toxins (10).

It must be stressed here that the information reported above about the pathogenicity of Candida organisms is derived either from clinical evidence in men and/or animals or from experimental infections in laboratory animals. There is a great lack of knowledge about the consequences of animal or human exposure to a "high" number of viable yeast cells or to repeated exposure to these cells. There is no doubt, however, that the continuous inhalation and/or ingestion of huge amount of viable yeast will affect the host immune system and may give raise to serious hypersensitivity reactions considering the strongly antigenic nature of the Candida cell surface.

Based on the above evidence, it may be concluded that the use of some Candida yeast strains for manufacturing single cell

proteins as animal feeds may pose serious risks not only to (i) workers (if exposed to viable yeast cells in production plants or during preparation and/or administration of feeds) and (ii) population groups (if exposed to emissions of viable yeast cells from manufacturing plants), but also to (iii) consumers of animal products containing viable yeast cells, (iv) farm animals exposed to viable yeast cells through contamination of the environment. Therefore, particular strains of Candida yeasts should not be permitted for industrial use in SCP manufacturing unless sufficient data are available to show that the strain employed is non-pathogenic and/or does not elicit serious hypersensitivity reactions.

2.1.2. Specific considerations relating to Candida strains used for manufacturing Toprina (*) and Liquipron (*)

C. lipolytica, strain No 246, and Candida sp., strain No ATCC 20.275 is cultivated on n-alkanes for manufacturing Toprina (*) and Liquipron (*) have been tested for pathogenicity in several laboratory animal species. The results obtained showed that the two strains did not proliferate but exhibited a tendency to persist especially in the kidneys of infected animals. This behaviour was, however, not significantly different from that of non-pathogenic C. utilis strains chosen as reference organisms. No data are available on the effects of the ingestion of Toprina (*) and Liquipron (*) on micro-organisms of the flora of the alimentary tract and on the colonization of pathogens in the alimentary tract.

Consequently, to protect consumers of animal products, workers involved in the preparation and administration of SCP-containing feeds, farm animals fed SCPs and the environmental organisms that might be exposed through contamination of the environment from mycoses and mycoallergies, Toprina (*) and Liquipron (*) should not be

permitted as animal feeds unless, in addition to the qualification of non-pathogenicity referred to in the previous section, they are also proven to be free of viable cells by a sensitive recognised method, the limit of detection of which is defined.

2.2. Biological and toxicological aspects

The biological effects of incorporating Toprina(*) or Liquipron(*) in the ration have been examined in a number of target species. The lipids of these products and probably of other Candida products from yeasts grown on n-alkanes contain a much higher percentage of odd-carbon-atom-number fatty acids, mainly C_{17}, than the usual dietary lipids. Investigations of the effect of the presence of these fatty acids in Toprina (*) and Liquipron (*) showed a dose-dependant accumulation of these fatty acids in the lipid fraction of all tissues as well as in milk and eggs in target species. Accumulation was greatest in the adipose tissue and involved mainly C_{15} and C_{17} fatty acids. Young poultry accumulated these fatty acids faster than older birds, while eggs accumulated more than body tissues. Similar accumulation was detected in the adipose tissue of mice, rats and monkey. These fatty acids were also present in the lipid fraction of brain, heart, liver and blood platelets. Accumulation usually reached a plateau after about 2 months feeding indicating steady state kinetics (1, 5, 29, 25).

Residues of n-alkanes ranging from 0.1 to 0.4% were detected in several samples of Toprina (*) and Liquipron (*) (9, 30). Investigations carried out on many target species have shown that n-alkane residues were also present in the adipose tissue and muscle of animals fed yeasts grown on n-alkane substrates. Accumulation levels reached a plateau usually within two months of continuous feeding of these yeasts in the ration (1, 6, 18, 26).

Subchronic feeding studies in rats, using levels up to 30% Toprina (*) or 20% Liquipron (*) in the diet showed no adverse findings (8). These studies included two 90-day studies with Toprina (*) and an 11 months study with Liquipron (*).

Lifespan studies were also carried out with both products. Two rat studies extending over 104 weeks and a mouse study lasting over 78 weeks were performed with Toprina (*) given at levels up to 30% in the diet. Apart from minor organ weight changes in rats, confined either to one sex or not dose-related, no carcinogenic or other adverse effects were noted in either species. Growth was somewhat reduced in all mice on yeast diet (17, 20, 22, 27).

A lifespan study in rats extending over 30 months with dietary levels of Liquipron (*) up to 26% showed an increased incidence of lymphomas in females and less so in the males. The rise in tumour incidence in females appeared to be related to dose (13). However, in the absence of knowledge of the incidence of these tumours in historical controls and with no mutagenicity data available, it is not possible to interpret these findings. These may well be due to epigenetic mechanisms. A second study, extending over 17 months, at a dietary level of 20% produced kidney calcification as the only adverse finding (23). A 28 months study in mice at a dietary level of 30% produced calcification in the hearts and kidneys of males and the kidneys of females. The only adverse finding in a two year dog study with Liquipron (*) was a dose-related reduction in body weight gain. Monkeys fed 2% Liquipron (*) for 2 years showed no adverse effects apart from diarrhoea and soft stools (24).

Reproductive and teratological studies with up to 30% of Toprina (*) in rats showed no adverse effects due to treatment (9, 21, 28a). Similar studies in mice with up to 27% Liquipron (*) produced only slight growth depression at the

highest test level. In rats the same top dose showed some adverse effects on pre- and post-implantation as well as on litter parameters. Maternal behaviour towards the litter also showed a dose-related adverse change. Some delayed ossification was also noted. Embryotoxicity was also noted when 17% Liquipron (*) was administered to pregnant rabbits (14, 15, 12).

The only existing mutagenicity test is a dominant lethality assay with Toprina (*), which was negative (28b). Liquipron (*) has not been tested for mutagenicity. The relay toxicity studies on Toprina (*) and Liquipron (*) did not yield any meaningful results (11).

A series of special studies was carried out on pregnant rats using the lipid fraction extracted from Toprina (*) at a dietary level equivalent to 75% Toprina in the diet. Examination of the progeny revealed some disturbance in the early myelination pattern in the brain and delayed maturation in post natal development (7). Similar effects were also produced by feeding the corresponding odd-carbon-atom-number fatty acids. These fatty acids were found in the lipids of treated rats, the milk and the progeny, this being evidence of passage accross the placenta (2, 3). Tissue microsomes associated with cytochrome b_5 and cytochrome b_5 reductase induction also contained these fatty acids but the mitochondria were normal. In contrast rats treated with up to 16% Liquipron (*) for 3 months and longer showed no effects on hepatic microsomal enzymes, haematological parameters, motor activity, coordination and neurotransmission except for slight increases in acetycholine and 5-OH-indoleacetic acid, slight reductions in liver triglycerides and adrenal cholesterol (10, 4, 5).

3. Conclusions

In the light of these data the Committees did not consider it feasible to assess the risks for the consumer of the use of protein products obtained from Candida yeasts cultivated on n-alkanes. The interpretation of some of the experimental findings was rendered difficult by the lack of certain basic data. In particular, information is missing on :

(a) the mutagenic activity of the relevant fractions of both Toprina (*) and Liquipron (*) biomass in _in vitro_ systems, with and without metabolic activation, and in _in vivo_ systems,

(b) the dose levels of lipids, extracted from these yeast products, which on ingestion do not produce any neurobehavioural and/or neuropathological changes in the progeny of treated laboratory animals,

(c) the dose levels of lipids extracted from the milk and eggs of animals ingesting these yeast products in their feed, which do not result in neuropathological and neurobehavioural changes in the progeny of treated laboratory animals,

(d) and the classical toxicological effects of fatty acids containing linear chains of 15 or 17 carbon atoms.

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REPORT ESTABLISHED JOINTLY BY THE SCIENTIFIC COMMITTEE FOR ANIMAL NUTRITION AND THE SCIENTIFIC COMMITTEE FOR FOOD ON THE USE IN ANIMAL NUTRITION OF PROTEIN PRODUCTS OBTAINED FROM BACTERIA OF THE METHYLOMONADACEAE FAMILY

Opinion expressed 25 September 1985

TERMS OF REFERENCE (October 1983)

The Scientific Committee for Animal Nutrition and the Scientific Committee for Food are requested to give their opinion on the following questions :

1. Do the products obtained from bacteria of the family of Methylomonadaceae and, in particular, from Methylophilus methylotrophus cultivated on methanol (Pruteen) (*) have a nutritional value for animals because they provide nitrogen or protein ?

2. Can the use in animal nutrition of products obtained from these bacteria and, in particular, from Pruteen (*) result in risks for human (consumer or user) or animal health, or be prejudicial to the environment ?

BACKGROUND

In accordance with the provisions of Council Directive 82/471/EEC of 30 June 1982 concerning certain products used in animal nutrition (**), Member States may, until such time as a Community decision has been taken, maintain authorizations granted within their territories before the date of application of the Directive concerning in particular products not listed under the product group indicated in Section 1.1. (Bacteria) of the Annex to the Directive.

(*) Registered trade name.
(**) OJ No L 213, 21.07.1982, p. 8

In accordance with the established procedure, the Commission consults the Scientific Committee for Animal Nutrition and the Scientific Committee for Food before producing a draft of the Community measures to be adopted for the compounds concerned.

OPINION OF THE COMMITTEES

Introduction

The Committees draw the Commission's attention to the fact that the term "Methylomonadaceae" has been replaced recently by the term "Methylococcaceae" (cf. Bergey's Manual of Systematic Bacteriology, vol. 1, Williams and Wilkins, Baltimore/London 1984). This family of bacteria includes various groups of rods, vibrios and cocci having the common characteristic of being able to utilize methane as their sole source of carbon and energy in earobic conditions.

Several bacteria of the family can be cultivated to produce edible bioproteins. However, the only exhaustive data on performance in livestock feeding at present available to the Committees were those concerning Pruteen (*), a bioprotein produced by Imperial Chemical Industries PLC, the dossiers of which were supplied. Pruteen (*) is obtained by growing Methylophilus methylotrophus (synonyms : Pseudomonas methylotropha, Methylomonas methylotropha), strain NCIB 10.515, on methanol. (Talbot C.J. et al., 1980).

In the early manufacturing process Pruteen (*) was not treated with hydrogen peroxide. The present procedure, which makes use of the treatment of the biomass with hydrogen peroxide before final dessication, was initially introduced as a bleaching measure. This treatment results in a partial oxidation of methionine to methionine sulphoxide and methionine sulphone, and cysteine and cystine to cysteic acid without major changes in the nutritional value of Pruteen (*). Of these conversion products, only methionine sulphone and cysteic acid, which account for a small proportion, are not bioavailable.

The only biologically significant modification noted was that the peroxide treated product was better tolerated by the chicken. On the basis of their similarity data available on both products have been used in the global evaluation of the safety of presently manufactured material. This evaluation refers exclusively to the use of Pruteen (*) in animal feedingstuffs. The material specified in the submission is not intended for human consumption without further processing.

1. Nutritional value

Pruteen (*) is marketed in two forms : granules (particle diameter : 500 microns) and powder (particle diameter : 25 microns). Its average crude protein content (N x 6.25) is 72.0 + 2% for the granules and 70.3 + 2% for the powder. The product contains 12.5 g nitrogen/100 g dry matter, of which 19.3% is in the form of nucleic nitrogen (13.8 g nucleic acid/100 g dry matter). The nitrogen of the cell wall of the bacteria (muramic acid + diaminopimelic acid + glucosamine and ethanolamine) represents 2.7% of the total nitrogen. The true protein content is 54.7 g per 100 g dry matter.

The amino acid composition of the protein of the product is (g/16 g nitrogen) : lysine 5.7; threonine 4.7; methionine 1.9 and cystine 0.7. Methionine is the first limiting amino acid in the rat. The availibility of lysine, methionine and thryptophan, determined in chickens, is respectively 89, 90 and 97 % (Abbey et al. 1980).

The chemical score of this protein is 59 as against that of the egg, 44 for soya, 66 for skimmed-milk powder and 74 for good quality fishmeal. The biological value of this protein, determined in the rat, varies from 66.2 to 76.8%, depending on the experiment. The addition to the diet of 0.2% of methionine raises this value to 88.4%.

Since the average digestibility of this protein is 90 + 2% in the rat, pig, calf and poultry, the net protein utilisation (NPU) is at least 90% of the biological value. In the rat and pig, the NPU is 65-70%. This figure is very close to that of soya (71%) but lower than that of good quality fishmeal (80%).

The constitutive fat of Pruteen (*) is accounted for essentially by phospholipids. The content in total lipids after addition of soya oil in amounts varying according to the form of the product, is of 8.5 + 1.5% for the granules and 13.2 + 2.0% for the powder. The fatty acids are chiefly made up of palmitic and palmitoleic acids. Linear fatty acids with an uneven number of carbon atoms are present in small quantities. 9, 10-methylene hexadecanoic acid (C 17) is the only cyclopropane acid present. According to Steel et al. (1977), the level of this acid in granula Pruteen (*) is 0.25% of dry weight, i.e. 3.2% of the total fatty acid. No cyclopropene acid has been detected.

The mean digestibility of the fat is 93% in the pig, calf and poultry for levels of incorporation of 6.5-37.5 of the ration. Mean metabolizable energy content of the product fed to poultry is 14.38 kJ/kg (Bolton and Blair 1974).

Pruteen (*) has been tested for its efficacy in numerous trials (Vogt et al. 1975, Hinks 1977, Braude et al. 1977, Waterworth and Heath 1981, Lloyd 1983). 64 trials grouping 194.250 subjects were carried out on poultry; 33 trials grouping 4.782 subjects on pigs and piglets, 69 trials grouping 8.150 subjects on calves and lambs, and 16 trials grouping 109.000 subjects on fish. Performances equal and sometimes superior to those of fishmeal and soya at isonitrogenous levels were obtained in mammals and birds with rations containing 5-10% of the product and fish with rations containing 30%.

On the basis of the foregoing information, Pruteen (*) has a good nutritional value as a source of protein for feeding to pigs, calves, poultry and fish.

2. Evaluation of risks

2.1. Pathogenicity and hypersensitivity

The bacterial strain used for the production of Pruteen (*) can be differentiated from other methylotrophic strains of the family of Methylococcaceae by variations in the composition of its cell walls (phospholipids, hydroxylated fatty acids, etc.) and DNA, and by its sensitivity towards antibiotics. On the other hand, the strain presents similarities to other gram-negative organisms, particularly E. coli and Salmonella spp., in its cell-wall structure.

On account of the obligate methylotrophic properties of this strain, it is reasonable to assume that viable cells which may normally escape the fermenter are unlikely to present any pathogenic risks for man or animals. This view-point is supported by the results of experiments carried out on mice by i.p. injection of 10^{10}-10^{12} viable or dead cells. Animal mortalities induced by either viable or dead cells were very similar and both significantly lower than mortalities ob- served with known pathogenic bacteria such as E. coli, Salmonella spp. and Staphylycoccus aureus. Moreover, no microbial proliferation was observed in the peritoneal cavity and in the organs of mice treated with viable cells.

Immunological monitoring of workers exposed to Pruteen (*) during its production has not revealed any allergic reaction, although exposure to dust concentrations exceeding 10 mg/m^3 resulted in influenza type symptoms and conjunctivitis which were probably irritative in nature. These findings stress the importance of controlling dust formation during the production, distribution and use. This control has been achieved by adopting special precautions when granulating or grinding (e.g. the addition of soya oil and use of a room with forced ventilation).

From these data, it emerges that no significant risks exist for the health of workers involved in production, distribution and use of Pruteen (*) if adequate precautions to prevent exposure to dust are taken.

2.2. Biological and toxicological aspects

2.2.1. Effects in target species

Feeding studies in pigs extending over 12-20 weeks with untreated or hydrogen peroxide treated Pruteen (*) used levels ranging from 2.25-30% in the diet. Growth rates and feed intake were improved but blood uric acid levels increased temporary at the 6.5% level. At higher levels of incorporation, feed intake, growth, kidney weights, liver weight, and adrenal weights were reduced. A 6-litter three generation study showed no pregnancy or litter abnormalities. A teratology study using levels of feeding from 8-18% did not show adverse effects. The 98-day feeding study with the treated-product showed slightly increased kidney weights compared to controls at the 30% test level but no histopathological abnormalities associated with this finding.

Feeding tests in calves extending over 16 weeks with 15-25% untreated Pruteen (*) produced no significant toxicological effects, although weight gain and feed conversion were reduced. Further studies with hydrogen peroxide - treated Pruteen (*) showed essentially the same effects (Sedgman, Roy and Thomas, 1985; Sedgman et al., 1985).
Nine different feeding experiments were carried out on broilers, layers and breeding hens with untreated or hydrogen peroxide-treated Pruteen (*) administered at levels of 2.5 to 25%. Dose levels above 10% reduced growth and lowered haemoglobin. At the 25% level liver and spleen weights increased as did the activity of the serum transaminases.

The livers of female birds developed necrotic and granulomatous lesions with the untreated product. Of the avian species tested (chickens, turkeys, quails, ducks), only chickens developed these hepatic lesions.

In two 8-week studies in broilers and one study in layers, the hydrogen peroxide-treated product did not increase the incidence of hepatic lesions at the level of 25% in the diet. The reason for this effect of the treatment with hydrogen peroxide is not known. Egg production and hatchability were not affected.

Trout fed on Pruteen (*) at 10-30% in the diet showed no adverse effects.

From these data, it emerges that Pruteen (*) as it is manufactured at present carries no appreciable risks for animal health when added at a rate of up to 7% in the diet of pigs, 10% in the diet of calves and poultry, and 30% in that of fish.

2.2.2. Effects on the quality of animal products

The use of Pruteen (*) at the recommended levels of incorporation (cf. point 2.2.1.) and at higher levels over long periods (10-30% for 16-17 weeks in pigs, 20% for 20 weeks in calves, 30% for 20 weeks in trout, 7.5-30% for more than 50 weeks in chickens) hardly alters fatty acid composition in the tissues and products of target species. Under the proposed conditions of use, the presence of trace amounts of C 17-cyclopropane acid was detected by capillary gas chromatography in the adipose tissues of pigs (up to 0.10% of total fatty acids) and in the egg yolks (0.11% of total fatty acids). No trace of the acid was found in the adipose tissues of chicken, calves or trout (limit of detection : 0.02% of total fatty acids). In pigs, the small quantities of cyclypropane acid are eliminated slowly. In man, degradation by beta and omega oxidation is probable (Lindstedt et al. 1974).

A comparative study of the technological and organoleptic properties of meat and eggs obtained from animals fed with Pruteen (*) and from those reared on conventional protein sources showed no significant difference.

2.2.3. Effects in laboratory animals

Numerous nutritional studies provide evidence that this bioprotein is metabolized in the same way as conventional proteins. With regard to toxicity studies, traditional toxicological tests are difficult to carry out on proteins because of the nutritional imbalance induced at high levels of incorporation in the diet. For this reason, isonitrogenous control diets based on casein were frequently included in the studies.

Four 90-day studies in rats using doses from 3.7-30% of untreated Pruteen (*) and two 90-day studies using 15 and 30% of the hydrogen peroxide-treated product showed little in the way of toxic effects. Serum levels of urea and allantoin, and kidney weights were increased in most tests only at doses of 30%. The slight but consistent decrease in haemoglobin was still within the normal range. Corticomedullary nephrocalcinosis without alteration of the kidney function was noted in female rats at doses of 15 and 30% and in male rats at the dose of 30%. The peroxide-treated materials was of the same toxicity as the untreated one. Several multigeneration reproduction tests on the untreated material showed no significant effects on reproductive function or litter parameters nor were there any teratological effects at 15 and 30% dietary levels.

A two-year chronic toxicity/carcinogenicity study using 7.5, 15 and 30% of untreated Pruteen (*) in the diet showed the effects of high protein intake and ionic imbalance at the levels of 15 and 30%. No carcinogenic effect was noted.

Beagle dogs from 6 weeks to 1 year were fed on a diet containing untreated Pruteen (*) at 30% and 60% levels and on an isonitrogenous casein diet. With the different diets, enlarged kidneys with fatty infiltration were observed and interpreted as a result of the protein overload. No urinary calculi were found. In the males, there was no significant difference in incidence between the casein controls and test dogs; in the females, dogs fed 60% Pruteen (*) had heavier kidneys than those which had been fed the isonitrogenous casein diet. 3 dogs out of 8 fed on a 60 % Pruteen (*) based diet and 1 dog out of 8 fed on a 30 % diet developed a transient weakness of hind limbs. Prolonged treatment produced hygromas of elbows and hocks. No cause could be identified. Among the species studied, only the dogs were affected and these effects appeared to be specific. One single experiment was done in Dalmatian dogs; 50 % Pruteen (*) diet raised serum urate.

The product was also submitted to mutagenicity tests. A dominant lethal test of untreated Pruteen (*) in mice using 15 % and 30 % in the diet, but with only 5-day male exposure, showed no mutagenic potential. A S. typhimurium reversion test, including S 9 metabolic activation, on a neutralised alkaline solution of both peroxide-treated and untreated material and of an in vitro enzymatic digest, and a mouse micronucleus test with the treated material also showed negative results. A host-mediated assay on hydrogen peroxide-treated product in mice with S. typhimurium TA 1537, TA 98 and TA 100 was inconclusive.

No mutagenic, carcinogenic or teratogenic effects have been observed in the studies reviewed with the product as prepared at present. No other adverse effects have been noted in laboratory animals if fed up to 7.5 % in the diet, provided the nutritional balance is observed.

3. Conclusions

In the light of available information, most of which is presented in this report, the Committees express the following opinion :

3.1. Of the protein products obtained from methylotrophic bacteria intended to be used as a source of protein in feedingstuffs, only Pruteen (*) has so far been the subject of a dossier forwarded to the members of the Committees and prepared in accordance with the "Guidelines for the assessment of certain products used in animal nutrition" (a). This opinion of the Committees is therefore limited to the assessment of this product.

3.2. In reply to question 1 of the Commission, the product examined has a good nutritional value as a source of protein for feeding to pigs, calves, poultry and fish.

3.3. In reply to question 2 of the Commission the product examined

- carries no appreciable risks for livestock if the level of incorporation does not exceed 7 % in the ration of pigs, 10 % in the ration of calves and poultry, and 30 % in that of fish,

- poses no appreciable risks for the health of workers involved in its production, distribution and use if adequate precautions are taken to prevent exposure to dust,

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(a) OJ n° L 126, 13.05.1983, p. 23

- carries no appreciable risks for the consumer from the consumption of products obtained from animals fed with a diet containing it. In addition, the characteristics and organoleptic properties of such products are not different from those of products obtained from animals reared on conventional protein sources,

- which originates from non-pathogenic bacteria, has no toxicological effects and is free from any residues of the culture medium or harmful contaminants. Its use in animal feed does not result in appreciable risks for the environment.

3.4. The Committees cannot give an opinion on other products obtained from bacteria of the family of Methylomonadaceae even if cultivated under the same conditions as Pruteen (*), without assurance as to the equivalence of the characteristics of such products.

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