The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines Evaluation Unit

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FURAZOLIDONE

SUMMARY REPORT

1. Furazolidone is a nitrofuran derivative used in the prophylactic and therapeutic treatment of infections caused by bacteria (Gram positive and Gram negative) or protozoa (e.g. *Histomonas meleagridis, Isopspora belli, Ballantidium coli*) in poultry, cattle (calves), pigs, rabbits and fish.

2. The nitrofurans, including furazolidone, have been previously evaluated by the CVMP in 1989 and 1993. In 1993, the nitrofurans as a chemical class were placed into Annex IV of Council Regulation (EEC) No 2377/90 with the exception of furazolidone. The provisional MRL for furazolidone was prolonged for an unrenewable period of 2 years until July 1st 1995. In 1993, an ADI could not be established because of the limited information about mutagenic and tumorigenic properties of furazolidone and/or its metabolites and the bioavailability and toxicological properties of bound residues. The sponsor was asked to provide i) data on the mutagenicity of the metabolite(s), ii) an oral 3-month study and iii) any other information to prove that the residues of furazolidone are neither genotoxic nor carcinogenic.

   In 1995 new study reports on mutagenicity (GLP standard) of the market residue 3-amino-oxazolidone-2, on the subchronic toxicity of furazolidone in dogs and rats, on residue depletion in pigs, bioavailability of residues, and on residue analysis were submitted.

3. 3-Amino-oxazolidone-2 was tested in the standard *Salmonella*/microsome assay in absence or presence of an S9-mix activating system (100-500 µg 3-amino-oxazolidone-2/plate). The trial was conducted using tester strains TA 98 and TA 1537 (indicative of frameshift mutations) as well as TA 100 and TA 1535 (indicative of base-pair transformations). Negative results were obtained in TA 98 and TA 1537 (when S9-mix was used) and in TA 100 (without S9-mix). All other experiments yielded positive (TA 1535 with and without S9-mix, TA 100 with S9-mix) or slightly positive results (TA 1537 and TA 98 without S9-mix). It was concluded that the test substance was mutagenic in this test system.

   Slightly positive results ere also obtained in an *Escherichia coli*/microsome plate test using the same concentration range as for the *Salmonella*/microsome assay, in absence or presence of an S9-mix activating system.

   The genotoxic potential of 3-amino-oxazolidone-2 (1000-5000 µg/ml) was also investigated using a human peripheral lymphocytes test. In absence of S9-mix, the test substance produced a statistically significant increase in chromosomal aberrations at all concentrations tested while in presence of S9-mix no effects were observed. It was concluded that 3-amino-oxazolidone-2 is a clastogenic substance under the conditions of this test.

   Two micronucleus tests in mice were performed with 3-amino-oxazolidone-2. In one test, male and female animals were treated with a single intraperitoneal dose of 500 mg/kg bw and 1000 mg/kg bw, respectively. A significant increase in micronucleated polychromatic erythrocytes was seen in animals of both sexes.

   In the second experiment, males received single intraperitoneal doses ranging from 32-500 mg/kg bw and females doses ranging from 250-1500 mg/kg bw. A statistically significant increase in the number of micronucleated polychromatic erythrocytes was observed in males of the highest dose group at 48 hours but not at 24 hours post administration. Independent of the dose, the substance induced an increased in the frequency of micronucleated polychromatic erythrocytes in individual animals of both sexes in all dose groups. Based on these results it was concluded that 3-amino-oxazolidone-2 was mutagenic. Apparently there are sex related differences in the susceptibility to these effects of 3-amino-oxazolidone-2 accompanied by notable interindividual variations.

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3-Amino-oxazolidone-2 was tested for its ability to induce DNA alkylation in the liver of male mice treated with single intraperitoneal doses of 32-500 mg/kg bw. Four hours post administration, the concentration of 7-methylguanine was found to be slightly elevated in a non dose dependent manner when compared to the untreated controls. No increase in the frequency of O^6-methylguanine was noted. The results of this study suggest that 3-amino-oxazolidone-2 leads to aberrant DNA-methylation. The biological relevance of the results is unclear.

4. Based on the results of a dose-range-finding study, a 3-month oral toxicity study with 3-amino-oxazolidone-2 (added to the diet) at doses of 1, 3 and 6 mg/kg bw/day was performed in dogs. The substance showed clear evidence of toxicity at the mid and high dose level (e.g. anaemia, prolongation of thrombin time, increase in serum liver enzymes). Males of the 6 mg/kg bw group had increased serum triglyceride levels and increased liver weights. A dose of 1 mg/kg bw/day caused slight anaemia. In all treatment groups, a dose dependent cholestatis was observed. No NOEL could be derived from this study.

5. A dose range finding study in rats revealed clear signs of toxicity at oral doses ranging from 100-1000 mg/kg feed. Based on these results, dietary dose levels of 10, 50 and 100 mg/kg feed (corresponding to 1, 4 and 8,5 mg/kg/day, respectively) were used for a 3 month feeding study in rats. At 100 mg/kg feed, an increase in hepatocellular vacuolisation was seen as a sign of general degenerative effects of on the liver. A decrease in feed consumption and bodyweight were recorded in the animals of the 50 and 100 mg/kg feed groups. An anaemic effect was detected at the end of the study in the same two dose groups. In this study a NOEL of 1 mg/kg bw/day (10 mg/kg feed group) was established.

6. No NOEL which could serve as a basis for the establishment of an ADI can be derived from the toxicity studies since treatment related effects were observed at the lowest dose test (1 mg/kg bw/day, in the 3-month dog study). Also the main metabolite of furazolidone, 3-amino-oxazolidone-2, caused effects in all recently submitted mutagenicity tests (point and frameshift mutations in bacteria, chromosomal aberrations *in vitro* and clastogenic effects *in vivo*).

7. A total ^{14}C-furazolidone residue depletion study in pigs subjected to 0, 21 and 45 day withdrawal times, following a 14-day oral treatment at 16.5 mg/kg bw/day was submitted. The ^{14}C-furazolidone was a mixture of two radiolabelled molecules with the radiolabel incorporated into the oxazolidinone ring and into the imine carbon adjacent to the nitrofuran ring. At 0 and 21 days withdrawal, liver contained the highest residues following by kidney, muscle and fat. Total residues were substantially lower by 21 days withdrawal, but were still in the mg/kg range at 45 days withdrawal.

	0 day	21 days	45 days
liver	41.1 mg/kg	4.4 mg/kg	2.1 mg/kg
kidney	34.4 mg/kg	3.4 mg/kg	2.0 mg/kg
muscle	13.2 mg/kg	3.3 mg/kg	2.4 mg/kg
fat	6.2 mg/kg	3.0 mg/kg	1.9 mg/kg

8. The bioavailability of the bound tissue residues from the ^{14}C-furazolidone pig residue depletion study was determined in rats. Bile duct-cannulated rats were fed lyophilised samples of liver and muscle from pigs sacrificed at 0 and 45 days after the last treatment. The results have been compared to those from bile duct-cannulated rates administered ^{14}C-furazolidone, orally via a stomach cannula and by incorporation of the radiolabelled compound into normal diet or into control liver and muscle. The absorption of non-extractable radioactivity from lever and muscle following extraction of these tissues has also been evaluated.

   The absorption of radioactivity after administration ^{14}C-furazolidone in polyethylene glycol 200 to rats, as calculated from the total recovered in urine, bile and tissues, was 87% of the dose. No marked change in the extent of absorption of radioactivity after doses of ^{14}C-furazolidone-medicated diet and muscle (90% and 96% of the dose respectively) was detected. After administration of ^{14}C-medicated liver, 73% of the dose was absorbed and the proportion of radioactivity excreted in bile was lower.

Less radioactivity was absorbed after administration of pelleted tissues from pigs from the ^{14}C-furazolidone residue depletion study to rats. Little of the absorbed radioactivity was excreted in the bile, most being recovered from urine and tissues. After oral administration of 0 and 45 days withdrawal liver, means of 40% and 19% of the dose respectively were absorbed. After oral doses of 0 and 45 days withdrawal muscle, means of 37% and 41% of the dose respectively were absorbed and in the latter case a greater proportion of the absorbed radioactivity was detected in the tissues.

The extraction of muscle tissue with organic solvents at 0, 21 and 45 days withdrawal led to the removal of 21.8, 18.6 and 13.7% of the total radioactivity respectively. The residue profiles in muscle are thus similar, with no apparent significant quantities of free metabolites. In liver, in contrast, 44 and 8.3% of the total radioactivity were extracted on days 0 and 45 respectively. After administration of the non-extractables 0 day and 45 day livers, means of 31% and 16% of the dose were absorbed.

9. 3-Amino-oxazolidone-2 is proposed as marker residue. The ratio of (released 3-amino-oxazolidone-2)/(total bound residues) was determined in pig livers from the ^{14}C-furazolidone residue depletion study. 3-amino-oxazolidone-2 could be released from 18% of the bound residues at 0 day withdrawal, and this fraction decreased to 8 and 6% after 21 and 45 days respectively. An analytical method for the detection of 3-amino-oxazolidone-2, released from protein-bound residues of furazolidone in pig livers, was validated with spiked and incurred tissues. No information is available for tissues other than liver.

10. No NOEL can be established from the newly submitted toxicity studies of furazolidone and, in particular, the main metabolite, 3-amino-oxazolidone-2, proved to be mutagenic in all investigated test systems. Additionally, furazolidone itself was previously shown to be mutagenic and cancerogenic in mice and rats. Total residues were still in the mg/kg range in all edible tissues. Forty five days after the last treatment bound residues were shown to be bioavailable in a rat study. 3-amino-oxazolidone-2 could be released from the bound residues in liver even after 45 days.

    Therefore it is proposed to include furazolidone into Annex IV of Council Regulation (EEC) No 2377/90.