COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

FLORFENICOL

SUMMARY REPORT (1)

1. Florfenicol, proposed for treatment of bovine respiratory disease also called shipping fever or transit fever, is a wide spectrum, synthetic antibacterial substance. It is structurally related to D (-)threo chloramphenicol (CAP), but differs from it in two fundamental aspects : firstly, presence of a p-methyl sulfonyl group instead of the p-nitro group, secondly, presence of fluorine atom instead of the hydroxyl group in the terminal primary alcohol function of CAP.

2. Florfenicol was shown to be like chloramphenicol in inhibiting peptidyl transferase activity specially on 70 S ribosomes. Selected bacterial strains highly resistant to chloramphenicol and thiamphenicol, because of chloramphenicol acetyltransferase production, were, in contrast, highly sensitive to inhibition by the fluorinated antibiotics.

3. Administered orally, florfenicol was not acutely toxic to mice and rats and no LD50 could be established (above 2000 mg/kg bw). After IP administration, the LD50 was close to 2000 mg/kg bw in rats.

4. Toxicity studies with repeated doses were conducted in mice (13 weeks), rats (7, 14, 28 days and 13, 52 weeks) and dogs (14, 28 days and 13, 52 weeks). Toxic effects reported for rats were changes in hematologic parameters and atrophy of testes. For dogs, an increase in liver weights was observed. The dog was the most sensitive species and the NOEL was 1 mg/kg bw/day.

5. In tolerance studies of florfenicol, carried out in cattle weighing between 161 and 210 kg, the main adverse reactions of animals revealed, other than local reactions, were a rapid decline in food and water consumption, reduced spontaneous activity, bodyweight loss and softening of faeces.

6. The results of a multi-generation study and of a 52 week study in rats revealed that oral doses of florfenicol had adverse effects on the male reproductive system with an NOEL of 1 mg/kg bw Several teratogenic studies were performed in mice (0, 1, 3, 60 mg/kg bw/day) and in rats (0, 4, 12, 40 mg/kg bw/day). High doses induced maternal effects and delayed ossification. The NOELs for maternotoxicity were 3 mg/kg bw/day for mice and 4 mg/kg bw/day for rats. florfenicol induced no foetal malformation at any dose level and showed no potential for embryo- or fetotoxicity.

7. The mutagenic or genotoxic properties of florfenicol have been studied by eight *in vitro* and *in vivo* tests. The CHO Chromosomal Aberration Assay showed an increase of chromosome aberrations at the highest dose (2500 µg/ml) and a slight increase of endoreduplication at 1250 and 2500 µg/ml in the presence of S9 mix. At 2500 µg/ml, severe cytoxicity (70% inhibition of cell growth) was observed and the test article precipitated in the test medium (625 µg/ml and higher). As the cytoxicity was higher than 50%, this positive response can not be taken into account. As *in vitro* tests for gene mutation in bacterial and mammalian cells systems were consistently negative, and since *in vivo* studies of the micronuclei and of the chromosome aberrations in bone marrow were also negative, it is concluded that Florfenicol is not genotoxic.

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8. Two carcinogenicity studies were carried out in mice (0, 20, 100, 200 mg/kg bw per day for 104 weeks) and in rats (0, 3, 12, 48 mg/kg bw per day for 104 weeks). In these two studies, the mortality was high (about 60%). In mice, two toxic effects attributable to treatment were described : the first one, on the male reproductive system in the mice exposed to 200 mg/kg bw, the second one, on the liver (benign hepatocellular tumours). For liver, the incidence of tumours was within that of the historical controls. However, in this study, the incidence in the concurrent group was unusually low (zero). In rats, the most obvious effects were observed for the testes : atrophy of the tubular epithelium, spermatogenic cells in the epididymis, an increased incidence of non-malignant interstitial cell tumours.

9. The potential for florfenicol to cause blood dyscriasias, such as aplastic anaemia, in humans was discussed in relation to the chemically related chemicals chloramphenicol and thiamphenicol. The data provided in support of a hypothesis that these molecules needed a nitro-group in order to cause the blood dyscrasias were insufficient in themselves to unequivocally prove that florfenicol did not have the potential to cause these effects. Human epidemiological data could not be used to demonstrate the safety of florfenicol as florfenicol has never been used in human medicine. Nevertheless, the working group concluded that it was highly unlikely that residues resulting from the veterinary use of florfenicol would cause serious blood dyscrasias in consumers.

10. The toxicological NOEL of 1 mg/kg bw, derived from the results of 52-week oral toxicity study in dogs would lead to a toxicological ADI of 0-0.010 mg/kg bw per day (i.e. 600 µg per person per day) after applying a safety factor of 100.

11. The sensitivity to florfenicol and its metabolites on the bacterial strains which constitute the human normal gut flora was tested by in vitro tests.

    The data available indicate that the metabolites of florfenicol have negligible microbiological activity compared to the parent compound florfenicol (florfenicol-amine approximately 90 times less active, florfenicol-alcohol approximately 30 times less active, florfenicol-oxamic not active at all). The only relevant microbiologically active residue is florfenicol. The bacterial population was comprised of 10 isolates of each of 10 genera/species representative of the human gut flora. As it was not possible to define the values for the safety factors CF1 and CF2, the microbiological ADI, based on the MIC-value for the most sensitive micro-organism (0.36 µg/ml for Fusobacterium sp) was established as follows :

$$\text{Microbiological ADI} = \frac{\text{MIC} \times \text{faecal bolus}}{\text{fraction of oral bioavailable dose to microorganisms} \times \text{average human bw}}$$

$$= \frac{0.36 \times 150}{0.3 \times 60} = 180 \text{ µg per person per day}$$

$$= 3 \text{µg/kg bw}$$

    As the microbiological ADI is lower than the toxicological one, the microbiological ADI was retained in order to establish the MRLs, that will be based on an estimate of the microbiological activity of residues.

12. The kinetic experiments using uniform-ring labelled 14C Florfenicol (the phenyl group was the site of the labelling) showed that the profile observed in the rat was similar, qualitatively, to that in cattle. In tissues, 5 metabolites were identified : florfenicol, florfenicol-amine, florfenicol alcohol, florfenicol oxamid acid, monochloride florfenicol. The mainly elimination route was via urine (63-71%). In cattle, after intramuscular administration, the fraction absorbed was 75%.

13. In a first total residue depletion study, it was shown that the highest concentrations were found in liver (4.1 µg of florfenicol equivalent/g, 30 days after treatment) and at the injection sites (0.5 µg of florfenicol equiv./g, 30 days after treatment). From the data on the metabolite composition in liver, kidney and injection site, it was shown that after withdrawal times of 15-30 days the microbiologically active florfenicol represented about ±5, ±35 and ±100% respectively of the metabolites. In a second depletion study, the residues were expressed as florfenicol-amine. In liver, the amounts of florfenicol-amine were 1.38 µg/g, 30 days post treatment. For this evaluation, the applicant has developed an analytical method, based on acid hydrolysis of tissue extracts, in order to change all the metabolites to florfenicol-amine.

14. Liver, kidney and muscle tissues are regarded as the target tissues. After hydrolysis of florfenicol and its metabolite (6N HCI, 2 hours, 100ºC), all the metabolites were converted into amine product. So, florfenicol-amine was selected such as the analytical marker residue.

15. There is an analytical HPLC method with UV detection technique proposed for monitoring of florfenicol-amine residues in the various tissues. The limit of quantification is 0.1 µg/g of florfenicol-amine for liver, muscle and kidney tissue. The limits of detection are respectively 0.045 µg/g, 0.058 µg/g and 0.07 µg/g for liver, muscle and kidney tissue.

16. The analytical method is based on the conversion of florfenicol and its metabolites to florfenicol-amine. MRLs were established on the basis of florfenicol-amine. For the conversion of florfenicol and its metabolites into florfenicol-amine, the following elements were taken into account :

    - the content of florfenicol in the various tissues (100% in muscle and fat, 35% in kidney, 5% in liver);
    - the ratio of molecular masses between florfenicol and florfenicol-amine (0.689).

    The MRLs expressed as florfenicol equivalent of 300µg/kg for muscle, 150 µg/kg for kidney and 200 µg/kg for liver became 200 µg/kg, 300 µg/kg and 3000 µg/kg respectively when expressed as florfenicol-amine.

    The target tissues were identified as muscle, liver and kidney and the following MRLs established.

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Florfenicol	Sum of florfenicol and its metabolites measured as florfenicol amine	Bovine	200 µg/kg 3000 µg/kg 300 µg/kg	Muscle Liver Kidney	