



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

METRONIDAZOLE

SUMMARY REPORT

1. Metronidazole belongs to the group of 5-nitroimidazoles. It is used in veterinary and human medicine for the treatment of infections with protozoa (*Trichomonas*, *Treponema*, *Histomonas*) and with obligatory anaerobic bacteria (*Bacteroides*, *Fusobacterium*, *Campylobacter*, *Clostridium*).

In pigs it is used for treatment of dysentery due to *Serpulina hyodysenteriae* at a dosage of 25 mg/kg/day for 4 days for therapy. For eradication of the disease in herds treatment for 7 days and a second treatment period of 5 days after 3-4 weeks is indicated. Metronidazole is intended for use in cows with retentio secundinarum in combination with neomycine administered as intra-uterine capsules. The dosage is 2.0 g metronidazole and 1.66 g neomycine per capsule and the recommended treatment regimen is twice during 48 hours.
2. The mechanism of action is explained by a partial reduction of the nitro group. Biological effects are produced via partially reduced metabolites which bind to bacterial and cellular macro- molecules. In bacteria the interaction between reactive metabolites and bacterial DNA inhibits DNA and protein synthesis and leads to the death of the microorganism. In humans and animals inter-action with cellular macromolecules and DNA were demonstrated. Single strand breaks of human DNA were observed following a single therapeutic dose with metronidazole. The same findings were confirmed in human lymphocyte cultures *in vitro*.
3. In rats orally administered metronidazole is rapidly and almost completely absorbed with maximum serum and tissue levels after 1-2 hours. 80% of the dose was absorbed after one hour. The distribution volume corresponds to that of total body fluid. Bactericidal concentrations corresponding to serum level were detected in cerebrospinal fluid, gall bladder, bones, and tissues of the pelvic regions. In mice metronidazole and its metabolites pass through the placental barrier and are distributed to all foetal organs and tissues. Metronidazole penetrates into milk, the concentration is about 50% of serum concentration. Serum-half life in rats is 11 hours after intravenous administration and 13.6 hours after intravaginal application. Excretion takes place mainly via the kidneys in urine but also via the gall bladder and through the intestinal wall with faeces.
4. The main metabolic pathway is oxidation of the two side-chains and conjugation of the parent substance and the metabolites. The percentage share of the individual metabolites varies from species to species. In man hydroxymetronidazole is the main metabolite with a share of 40-50%. In rats 97% of radioactivity excreted in urine can be attributed to nitroimidazoles with an intact ring structure. A further important metabolic pathway is the degradation of the compound via reduction of the nitro group and cleavage of the imidazole ring. Acetamide and N-(2-hydroxyethyl) oxamic acid are produced as final metabolites. In mammals the metabolism of 5-nitroimidazoles *in vivo* is related to nitro-reductase activity and oxygen tension in tissues. 5-nitroimidazoles lead to covalently bound residues with a persistent imidazole structure. The toxicological safety of these residues was not assessed.
5. Acute toxicity is low. Oral LD₅₀ values vary between 4350-5000 mg/kg/bw for mice, greater than 5000 mg/kg/bw for rats and greater than 750 mg/kg/bw for dogs. Intravenous LD₅₀ values for mice range between 250-1260 mg/kg/bw. For rats the intravenous LD₅₀ values lies above 100 mg/kg/bw but below 1575 mg/kg/bw.

6. Repeated dose toxicity studies were performed with various species.

Short-term repeated dose toxicity studies:

In rats during a 4-week oral medication study with doses of 25 or 50 mg/kg/bw per day, bodyweight and biochemical parameters were comparative with controls. The study design was not adequate and the observation period too short. Therefore the results cannot be accepted.

Medium-term repeated dose toxicity studies:

In monkeys two studies were performed with oral doses by gastric intubation. In the first study doses of 45, 100 and 225 mg/kg/bw were given for 14 weeks. Lack of appetite and bodyweight depression were found in all these groups and at the highest dose histological changes in the liver were observed. A NOEL cannot be derived from this study.

In dogs an oral study was conducted at dose levels of 75, 110, 150 and 225 mg/kg/bw for 17 weeks. All animals in the two highest dose groups died or were killed moribund showing ataxia, muscular rigidity, tremors and prostration. Similar symptoms were seen in animals at 110 mg/kg/bw but only one died. Ataxia and tremors were also seen in some dogs of the lowest dose group. A NOEL cannot be derived from this study.

In an oral feeding study for 18 weeks rats received 75, 150 or 300 mg/kg. The growth rate was reduced at all doses. At the highest dose increased liver/bodyweight and kidney/bodyweight ratios were observed and in males a reduction in testis weight and in spermatogenesis. No NOEL can be established.

Long-term repeated dose toxicity studies:

Feeding studies were conducted with CD-1 and CF-1 strain of ICR Swiss mice for 78 and 92 weeks respectively, at dose levels of 75, 150 and 600 mg/kg/bw/day. In the CD-1 strain at 75 mg/kg/bw decreased bodyweight and hypospermatosis in 26% of the males was observed. At 150 mg/kg/bw a decreased seminal vesicle/bodyweight ratio in males was additionally noted. At 600 mg/kg/bw also a decreased testes/bodyweight ratio, hypospermatosis in 53% and testicular atrophy in 23% of the males was observed and a decreased uterus/bodyweight ratio in females. The CF-1 strain showed at the lowest dose a decreased prostate/bodyweight ratio in males. At 150 mg/kg/bw a decreased heart/bodyweight ratio was observed and at the highest dose a decreased heart/bodyweight ratio was also seen in females. A NOEL cannot be established.

In an oral study in rats conducted for 80 weeks with 75, 150, 300 mg/kg/bw and 600 mg/kg in a separate group for 13 weeks, at 300 mg/kg depressed bodyweight was seen in all animals and additionally in males testicular dystrophy was regularly seen. At the lower doses blood parameters were changed. In animals receiving 600 mg/kg/bw per day, testicular dystrophy, prostatic atrophy and reduced growth rates were frequently seen. A NOEL cannot be established.

7. No tolerance studies were submitted. From a clinical trial study with the combination product metronidazole/neomycine in 112 postparturient cows it can be concluded that intrauterine treatment at therapeutic doses is tolerated without adverse effects. For oral use in pigs four times the therapeutic dose is stated to be well tolerated by weaner pigs. Side effects occurring seldom at therapeutic doses are oedema of eyelids, rectum and vulva that are fully reversible.
8. Reproductive toxicity was shown in long term studies in rats and mice, especially hypo-spermatosis, decreased prostate/bodyweight ratios and testis/bodyweight ratios were seen. A study concerning effects on spermiogenesis was conducted in boars. Four animals received a 50% overdose and two animals a 100% overdose. Sperma was collected up to 10 weeks after treatment two times a week. No significant changes with control animals were noted. The study was conducted in four animals only, not to GLP standards and the observation period was too short. Therefore the results cannot be considered as relevant with regard to the long term studies. No studies on fertility were conducted although the signs of toxicity especially on the male reproductive system indicate a possible influence on fertility parameters.

9. A study on foetotoxicity/teratogenicity in Swiss Webster mice at doses of 15 mg/kg/bw intraperitoneal on day 8, 10, 12, and 14 of gestation showed a significant increase in the number of dead and malformed fetuses. In an *in vitro* study with cultivated Sprague rat embryos metronidazole at concentrations of 2 mM showed low foetotoxicity (one abnormality/11 viable embryos). Teratogenicity was not sufficiently tested although there were signs of teratogenic effects in the few existing studies.

10. Metronidazole induces gene mutations in various microbial test systems and in fungi as well as mitotic gene conversion in yeasts. Under hypoxic conditions, chromosome mutations were observed in mammalian cells. Metronidazole triggers chromosomal mutations in the mouse *in vivo*. In indicator systems (UDS) genotoxic effects occur in primary hepatocytes of man and rats *in vitro*. Recent studies in man show a genotoxic mechanism *in vivo*. After a single treatment in the range of therapeutic doses metronidazole leads to DNA single strand breaks. Under *in vitro* conditions concentrations below the therapeutic plasma concentrations lead to genotoxic effects in human lymphocytes.

Additionally the two main oxidative metabolites have shown mutagenic effects. The mutagenic potential of hydroxymetronidazole is higher than that of the parent compound. According to the results of various studies and tests metronidazole is considered as genotoxic and mutagenic.

11. Additional data on the genotoxicity of metronidazole not present in the original application came to the knowledge of the Committee. The data included two *in vivo* studies in humans on the induction of chromosome aberrations in peripheral lymphocytes after oral administration of metronidazole and two “comet” assays on the induction of DNA damage in human peripheral lymphocytes.

Of the two human *in vivo* studies and the two “comet” assays one each indicated genotoxic effects of metronidazole while the other did not. However, the respective studies indicating that no genotoxic risk is associated with metronidazole contained data of insufficient statistical power to invalidate the results of the studies showing genotoxic effects of the substance.

Consideration of the available data on the genotoxic effects of metronidazole leads to the conclusion that, at the current state of scientific knowledge, metronidazole must be considered to have genotoxic effects and specifically genotoxic effects in humans *in vivo* after oral exposure.

12. Metronidazole shows carcinogenicity in mice and rats.

More recent studies with a 100-day-treatment period and lifetime observation in rats (30 mg/kg bw and day per gavage) and mice (2 mg/day, i.e. ca. 66 mg/kg bw and day per gavage) confirmed earlier results of lifetime feeding studies in rats and mice at higher dosages. The lowest investigated dose of 30 mg/kg/bw per day lies in the range of the therapeutic dose for man. At this oral dose in rats a significant increase in mammary tumours (fibroadenomas: 56%, in treated vs. 18% in control rats, adenomas: 36% vs. 16%, fibromas: 22% vs. 0%, carcinomas; 10% vs. 0%) was observed in females after a mean latency period of 100.5 weeks. In mice at the above dose malignant lymphomas were observed in females (44.1% in treated vs. 0% in control mice) and lung adenomas in males (66.6% in treated vs. 26.3% in control mice).

Metronidazole must be considered as a genotoxic carcinogen in animals. This view is shared by the International Agency for Research on Cancer (IARC) which classified metronidazole as “possibly carcinogenic to humans” belonging to group 2 Ab substances.

It was argued that the carcinogenic effect of metronidazole was the result of a tumour promoting rather than a tumour initiating action of the substance. However, no possible mechanism of action was proposed and no data on such a promoting mechanism were submitted.

13. No studies on immunotoxicity were provided.

14. The microbiological properties of metronidazole are known from its use in human medicine. Metronidazole is used for the decontamination of the human colon as prophylaxis of postoperative infections in patients undergoing colorectal surgery. The MIC value of metronidazole for most of the colonic obligate bacteria lies between 2-6 µg/ml. The relation to relevant bacteria in animals cannot be established. However in view of the mutagenic and carcinogenic potential no further data will be requested.
15. Metronidazole has been used in human medicine since about 30 years. The clinical use covers the treatment of anaerobic bacterial infections, amoebiasis, trichomoniasis giardiasis and Crohns disease. The doses vary according to indications between 250-800 mg/day for 5-7 days, up to 2 g in a single dose. For humans an oral dose of 180 mg/kg/bw is at the borderline of tolerance where severe nausea and vomiting were observed. Mostly metronidazole is used only for short term treatment. In man metronidazole administered orally is rapidly and almost completely absorbed. The bioavailability is 95%. $T_{1/2}$ after oral and intravenous administration is about 8,3 hours. The main metabolite is hydroxymetronidazole with a share of 40-50%. Mutagenic effects of metronidazole at therapeutic dose levels were demonstrated in men.
16. The information on the metabolism of metronidazole did not address the formation and toxicological relevance of covalently bound tissue residues with intact imidazole structure, which has been demonstrated for other nitroimidazoles.

No toxicological NOEL could be identified for metronidazole in repeated dose toxicity studies.

The influence of metronidazole on fertility has not been specifically tested, although impairment of male fertility was noted in the repeated dose toxicity studies.

The teratogenicity of metronidazole was not adequately tested, though its has been shown to have teratogenic potential.

Metronidazole has proved to be mutagenic in mammalian cell systems and human cells *in vitro* and in the mouse *in vivo*. Furthermore, genotoxic effects are known in man following oral treatment with metronidazole.

Metronidazole has proved to be carcinogenic in mice and rats. An increased incidence of tumours in very young patients undergoing long-term metronidazole therapy raised suspicion that metronidazole is potentially carcinogenic in man. According to IARC metronidazole is considered as a possible carcinogen in humans.

No data on a possible tumour-promoting mechanism of metronidazole are available.

Due to the genotoxic mechanisms of the carcinogenicity of metronidazole it will not be possible to establish a threshold level and calculate an ADI.

17. There was no full residue file and the pharmacokinetic data in the food producing animals: horse, cattle, and swine focused mainly on absorption and plasma elimination following the parenteral and oral route of administration of various pharmaceutical formulations.

There was no total residue study and data on metabolism in the target animals made available.

The 5'-nitroimidazoles are known to be rapidly metabolised. The main metabolite results from oxidation of the side-chain in the C-2 position of the imidazole ring. The residues are covalently bound to tissue proteins. For metronidazole no information is available on bound residues in the tissues of the target animals.

18. No information was given on the depletion of total drug-related residues and the ratio of the marker residues to total residues.

Few tissue distribution and excretion data were determined in pigs, but metronidazole residues could only be detected in plasma and urine. In all tissue samples, except for one fat sample, no metronidazole residues could be detected, but this could well be due to the method of analysis.

After intrauterine treatment of cows with the recommended dose, residues of metronidazole and its metabolite hydroxymetronidazole could be detected in the milk at 2 and 6 hours after the last application which decreased to below the limit of detection of the analytical method at 43 hours. But metronidazole and hydroxymetronidazole could not reliably determined as indicated by recovery experiments, nor were the limit of detection and limit of quantification of the analytical method given.

19. For the determination of metronidazole in pig tissues a combined method using thin-layer chromatographic separation and gas chromatography with electron capture detection was proposed. However, this method was not adequately described and validated with respect to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community.

Conclusions and recommendation

Having considered :

- insufficient data on repeated dose toxicity and all aspects of reproduction toxicity,
- the genotoxic properties of metronidazole and its two main oxidative metabolites,
- the proven carcinogenic properties of metronidazole in animals and its suspected carcinogenic effects in man, reflected by the IARC classification as a possible human carcinogen,
- the lack of data on metabolism in the target species,
- the insufficient information on residue depletion,
- the inadequate routine analytical method;

the Committee on Veterinary Medicinal Products recommends the inclusion of metronidazole into Annex IV of Council Regulation (EEC) No 2377/90.