



COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NOVOBIOCIN

SUMMARY REPORT

1. Novobiocin is an antibiotic consisting chemically of three distinct entities: the sugar noviose, a coumarin moiety and a benzoic acid derivative. The sub-entity consisting of noviose plus the coumarin moiety is referred to as novenamine, the sub-entity consisting of a coumarin plus the benzoic acid derivative is referred to as novobiocic acid. In veterinary medicine it is used for intramammary infusion treatment of bovine mastitis in lactating and dry dairy cows alone or in combination with other antimicrobials. A dose of 200 mg/quarter is recommended.

Novobiocin has also been widely used in human medicine.

2. Novobiocin is primarily a bacteriostatic compound, but shows bactericidal properties at high concentrations. Novobiocin is especially effective against gram-positive organisms such as *Staphylococcus spp.* and *Streptococcus spp.* It is resistant to β -lactamase enzymes.

No information is available on pharmacodynamic properties of novobiocin, other than bacteriostatic activity.

3. Pharmacokinetics studies have been performed in mice and dogs as well as in humans

Novobiocin is stable under acidic conditions, like those encountered in the stomach, and is rapidly absorbed from the gastro-intestinal tract. After a single oral treatment with 10 mg/kg bw in dogs, peak serum values were attained within 4 hours, whereas in mice undergoing an analogous treatment peak serum levels were achieved within an hour. The oral absorption in laboratory animals is around 30%.

Novobiocin is metabolised to a moderate degree in animals. Two hydroxylated isomers, an epoxide metabolite and conjugated metabolites of novobiocin have been isolated in both *in vitro* and *in vivo* experiments; however the parent compound is consistently the predominant molecule in both tissues and fluids.

Comparative *in vitro* studies have shown that metabolism in the dog, cow and human is qualitatively comparable.

The liver is the main target organ for novobiocin metabolism as well as the bile is the main excretion pathway. Hepatic/biliary excretion is very rapid, irrespective of the route of administration. Faeces are therefore the principal route of excretion for novobiocin and its metabolites. Only very small amounts are excreted in the urine. In dogs treated orally with a single 10 mg/kg bw dose, approximately 30% of the administered dose was excreted within 24 hours in the faeces, while only 1% of the dose was excreted within 48 hours in the urine.

Novobiocin has a pKa value of 4.3 (and it is therefore predominantly ionised in plasma and milk), is highly bound to plasma albumin (at 5 to 30 mg/ml: 92% in cattle, and at 100 mg/ml: 99.2% in man), to milk (15 to 28%) and to udder tissue homogenates ($\pm 40\%$), has a relatively small apparent volume of distribution ($V_d \pm 0.3$ l/kg), and has a relatively short elimination half life (± 1.5 hours in cattle). Novobiocin diffuses into pleural fluid, ascitic fluid, joint fluids and milk in concentrations lower than those in plasma. For example, 50 minutes after intravenous administration injection of 10 mg/kg novobiocin to dairy cows, C_{max} in milk was 1.94 mg/ml *versus* 41.3 mg/ml in plasma.

Pharmacokinetic studies in the cow have shown that, after intramammary infusion, novobiocin is quickly absorbed from the udder and then metabolised by the liver into several metabolites before being rapidly excreted in the bile and faeces, primarily as parent drug. Five metabolites have been identified in the cow: two hydroxylated isomers, an epoxide metabolite, a sulphate conjugate and a glucuronide conjugate. A sixth secondary metabolite (carboxylic acid derivative of hydroxylated novobiocin) has also been isolated only in cow faeces. About 2% and about 60% of the total dose was excreted in the urine and in faeces. In urine, the parent compound was the only compound identified. In faeces, the parent compound accounted for about 30% and the 6 metabolites for 28% (the oxidated metabolite 15% to 22%, the sulphate conjugate, 7% to 15%, the other four metabolites 10% in toto). In lactating animals about 20% of the total dose was excreted in the milk, all as parent novobiocin.

4. The oral LD_{50} values for mice and rats were approximately 1000 mg/kg bw and 3200 mg/kg bw, respectively. Intraperitoneal LD_{50} values ranged from 260 to 370 mg/kg bw for mice and rats; while for guinea pigs the value was 11.5 mg/kg bw. Studies were conducted in the mouse with novobiocin-containing mastitis formulations sterilised by ^{60}Co irradiation: calculated LD_{50} values for novobiocin ranged from 600 to 800 mg/kg bw.
5. In an old 90-day dietary study in mice, a dose-related decrease of ovarian weight was observed at dose levels equivalent to 300 mg/kg bw and higher, with other toxic effects observed at 1000 mg/kg bw; the NOEL was 100 mg/kg bw.

In a second 90-day mouse dietary study in mice no treatment-related effects up to the top dose level of 2100 mg/kg food, equal to 300 mg/kg bw/day.

In a dietary eight-weeks study no treatment-related effects were seen in rats up to the top dose level, equal to 385 mg/kg bw/day.

In a 60-day oral study in dogs treated orally (gelatin capsules) with 0, 30, 100 or 300 mg/kg bw/day, a gross orange discoloration of the colonic mucosa was observed at the top dose level; altered urinary parameters (albuminuria, increased retention of bromosulphalein and phenolsulfonphtalein) were also seen at 300 mg/kg, but the low number of animals (two females and one male per each test group) prevented the performing of a meaningful statistical analysis. A dose-related incidence of vomiting was seen at 100 mg/kg and higher, with no cases occurring at low-dose and in controls. Therefore a NOEL of 30 mg/kg bw/day was retained.

6. In a pre-GLP 3-generation (two litters/generation) study, novobiocin was administered via the diet to three groups of rats at 0, 10, or 20 mg/kg feed, equivalent to approximately 0, 1, and 2 mg/kg bw/day. A satellite prenatal toxicity study on F3b litters was incorporated as part of the 3-generation reproduction study: in this study dams and sires of the P2 generation were treated at 20 times the above doses (i.e. 0, 20 or 40 mg/kg/bw day) starting 2 weeks prior to breeding for females and 6 weeks for males. No effects were observed on fertility or litter parameters up to the highest dose levels tested, i.e. 2 mg/kg bw and 40 mg/kg bw, respectively. It was concluded that novobiocin is devoid of any significant potential to affect fertility or intrauterine development.
7. Novobiocin inhibits bacterial DNA-gyrase, which in turn shows DNA-replication. Much higher (1000 fold) concentrations are needed to produce similar inhibition of the equivalent enzyme (topoisomerase II) in mammals cells. Novobiocin also inhibits repair DNA synthesis in certain human cells *in vitro* (lymphocytes and fibroblasts), whereas other cells are resistant (human keratinocytes, Chinese hamster ovary (CHO) cells). The effects on excision repair are due to a non-specific effect on ATP metabolism.

A number of *in vitro* studies have been published on the possible genotoxicity of novobiocin. Novobiocin did not induce gene mutations in bacteria (*Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, TA 102 and TA98, both with and without metabolic activation; and *Escherichia coli*). No mutagenic effects were observed on L5178 mouse lymphoma cells at the tk-locus. Novobiocin did not interfere with DNA repair in *Bacillus subtilis*.

Several studies were performed on the cytogenetic effects in Chinese hamster V79 cells. Novobiocin induced chromatid-type aberrations. No increase of chromosome aberrations was induced by novobiocin in human peripheral blood lymphocytes. Novobiocin induced sister chromatid exchange in human peripheral blood lymphocytes and in one of the studies performed on V79 cells. Unscheduled DNA synthesis (UDS) was increased in rat thymocytes, but reduced in rat splenocytes.

It is concluded that novobiocin can interact with DNA synthesis and repair and cause clastogenicity, sister chromatid exchange and unscheduled DNA synthesis in certain cell types. However, there is no evidence that novobiocin can damage the DNA or cause gene mutations.

No *in vivo* studies were available. Therefore it remains unclear whether the genotoxic effects seen *in vitro* are expressed *in vivo*.

8. In a 25-month feeding study, groups of 50 male and 50 female Sprague-Dawley rats were fed sodium novobiocin at dietary concentrations equivalent to doses of 0, 1 and 2 mg/kg bw/day. The rats were derived from the F1 generation of a 3-generation reproduction study in which their dams and sires had been given the same dietary doses throughout breeding, gestation and lactation. The number of animals with mammary fibroadenomas was statistically significantly increased in females given 1 mg/kg bw/day, but not in males or in females given 2 mg/kg bw/day. No other types of tumours were significantly increased in numbers. It was concluded that sodium novobiocin was not carcinogenic in rats.

9. When tested in an *in vitro* assay on an adequate range of bacteria representative of the human gut flora (overall 100 strains tested), the minimum inhibitory concentrations (MIC₅₀) of the most sensitive genus assayed (*Lactobacillus* and *Bifidobacterium*) was 0.25 µg/ml.

Against the most sensitive anaerobic bacteria found in the gastrointestinal tract of humans, the MICs of novobiocin metabolites were consistently higher (4 to 32-fold) than the MICs of parent novobiocin. Therefore, the antimicrobial activity of the metabolites is much lower than that of the parent compound.

10. The effect of the presence of various concentrations of novobiocin in milk on the performance of bacterial starter cultures used in yoghurt, cheese and buttermilk/sour cream production using a starter culture growth assay has been determined. No adverse effects on cheese and yoghurt production were observed at a concentration of 0.39 µg/ml.

11. No specific studies on immunotoxicity have been performed.

No alterations of target organs or haematological parameters relevant to immune function have been observed in repeated-dose toxicity studies. Novobiocin can cause hypersensitization reactions in human patients.

12. Novobiocin was originally developed for oral use in humans with a recommended dose of 1000 mg/person/day to 2000 mg/person/day (approximately 16.6 mg/kg bw to 33.3 mg/kg bw).

In human patients, the pharmacokinetics of novobiocin shows no significant differences as compared to laboratory animals. The drug is rapidly absorbed with peak serum values 2 to 4 hours after oral administration at a therapeutic dose range. Novobiocin binds to serum proteins and re-enters the bowel via enterohepatic circulation.

Side effects at the recommended therapeutic dose included transient loose stools or diarrhoea. However, no significant potential to cause more severe gastrointestinal disturbances has been reported, following widespread therapeutic usage. Hyperbilirubinaemia was observed in infants treated with 50 mg/kg bw. The most common adverse effect of therapeutic use of novobiocin in humans is skin rash, regarded as a hypersensitivity phenomenon. Typically, such skin rashes are erythematous, urticarial, maculopapular, or scarlatiniform and occur following at least one week of therapeutic use in 7 to 20% of individuals treated. Only in a few instances have such rashes been reproduced by re-administration of novobiocin, and the contribution of other co-administered drugs could not be excluded in many instances. The incidence of skin rashes also appears to be dose related as it is greatly reduced when patients were administered 1 g novobiocin daily for 12 days (33.3 mg/kg bw) compared with doses of 2 g daily for more than 6 days (66.7 mg/kg bw). However, hypersensitivity reactions were observed in patients treated orally with doses as low as 20 mg/kg bw for 10 days or more. The available evidence suggests that dose levels lower than 10 mg/kg bw are unlikely to pose a significant hazard of hypersensitivity reactions in humans. There are no reports of anaphylaxis following therapeutic use in humans. A NOEL could not be identified in humans.

13. Having considered that although it remains unclear whether the genotoxic effects seen *in vitro* occur in life and that no carcinotoxicity was reported in rats, a toxicological ADI of 0.020 mg/kg bw (i.e. 1.2 mg/person) was established based on the NOEL of 2 mg/kg bw/day observed in the 3-generation study by applying a safety factor of 100.
14. For the assessment of the microbiological risk, use was made of the formula that was recommended by the CVMP:

$$\text{ADI} = \frac{\text{MIC}_{50} \text{ for the most sensitive organism} \times \text{CF2}}{\text{CF1}} \text{ (}\mu\text{g/ml) } \times \text{daily faecal bolus (150 ml)}$$

$$\frac{\text{fraction of an oral dose available for micro-organisms}}{\text{weight of human (60 kg)}}$$

(μg/kg bw)

Based on the above formula and on *in vitro* data for inhibition of human gut flora, the microbiological ADI can be calculated as follows:

$$\text{ADI} = \frac{0.25 \times 2}{1} \times 150 = 1.25 \mu\text{g/kg bw i.e.} = 75 \mu\text{g/person}$$

$$\frac{1.0 \times 60}{1}$$

The following assumptions were made:

- CF1 = 1 because the most sensitive MIC₅₀ was used in the calculation and there is no evidence of chromosome- and plasmid-mediated resistance ;
 - CF2 = 2 for the effect of inoculum density on MICs taking into account that the MICs values increased by two factor according to the size inoculum ;
 - 150 g was the weight of the daily faecal bolus;
 - 0.25 μg/ml = the MIC₅₀ of the most sensitive genus assayed (*Bifidobacterium*);
 - 1.0 = as there is an enterohepatic cycle so that the total administered compounds is almost completely excreted in faeces.
15. Several milk and tissue residue studies have been performed with a variety of different formulations containing novobiocin. Many of these residue studies were carried out with novobiocin in combination with other antibiotics, e.g. penicillin. Only microbiologically active residues were measured using conventional microbiological plate assays. In both milk and tissues the limit of detection for the microbiological active residues of these methods was in the order of 100 μg/kg. The pharmacokinetic characteristics of these antibiotics are considered not to significantly influence the kinetics of novobiocin.

In one series in lactating cows, the residues in edible tissues were always below 100 µg/kg, 96 hours post dosing (no information was available on earlier slaughtering points). No residues of novobiocin could be detected in most of the samples collected 60 hours after treatment. In the 3 studies performed in dry cows no residues were detectable in edible tissues collected approximately 30 days after treatment.

A second series of milk and tissue depletion residue studies has been conducted utilising an HPLC analytical method. The milk depletion study showed that, after treatment of all four quarters of 16 cows (8 high yielding cows and 8 low producing cows) with a dose of 150 mg novobiocin/quarter twice with a 24-hour interval (maximum treatment regimen), the depletion of novobiocin in milk was very rapid. At the first milking after the second infusion of the mean novobiocin milk concentration was 11 525 µg/l (range: 3901 to 3980 µg/l), at the second milking the mean novobiocin concentration was 776 µg/l (range: 96.6 to 3204 µg/l), at the third milking the mean novobiocin concentration was 93.2 µg/l (range: less than 20.1 to 438 µg/l). At the fourth milking, residues of novobiocin could not be quantified in high yielding cows, all concentrations being below the limit of quantification (20 µg/l). The low yielding cows had milk residue concentrations of novobiocin ranging from less than 20 µg/l to 71 µg/l. At the fifth milking (58 hours) after last infusion, no novobiocin residues were quantifiable (below the limit of quantification).

In the tissue depletion study 20 lactating cows were treated with 150 mg of novobiocin in each of all four quarters twice at a 24 hour interval. Four cows were slaughtered at each of five time points after the last dose: at 12 hours, 1 day, 2 days, 4 days and 8 days. HPLC analysis for parent novobiocin residue in edible tissues found quantifiable concentrations (above the limit of quantification of the method of 36 µg/kg) at 12 hours only in liver (3 of 4 cows with residue concentration of 42, 70 and 49 µg/kg respectively), kidney (1 of 4 cows: 50 µg/kg), fat (1 of 4 cows: 58 µg/kg). Trace levels of two metabolites (one hydroxylated isomer and the epoxide at concentration of approximately 20 µg/kg) were also found in liver. Parent novobiocin residue was not detected in muscle at any time point (limit of detection of 18 µg/kg). No edible tissue had measurable concentrations of parent novobiocin by 24 hours post last treatment, neither any novobiocin metabolites were found at detectable levels at this time in either liver or kidney tissue.

16. The residue studies show that after intramammary administration, the parent compound was the only residue in milk. In the edible tissues at 24 hours after intramammary administration no residues of novobiocin or its metabolites were found.

Thus novobiocin can be considered the marker residue in milk and there is no need to consider the ratio of marker residue (novobiocin) to total residue in both milk and tissues.

17. A fully validated HPLC method according to the requirements of Volume VI of the Rules Governing Medicinal Products in the European Community is available for milk with a limit of detection of 15.7 µg/l and a limit of quantification of 20 µg/l. The specificity with respect to the presence of lincomycin, neomycin, polymyxin B, dihydrostreptomycin, ceftiofur and penicillin is established.

An analytical method for monitoring of novobiocin in tissues is also available.

Conclusions and recommendation

Having considered that:

- an ADI of 1.25 µg/kg bw (i.e. 75 µg/person) was established,
- the parent compound is the marker residue in milk and represents 100% of total residues in milk,
- after intramammary administration, in lactating cows no residues of novobiocin or its metabolites were found in the edible tissues at 24 hours,
- the analytical method for milk is fully validated;

the Committee for Veterinary Medicinal Products recommends the inclusion of novobiocin for bovine milk in Annex I of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Novobiocin	Novobiocin	Bovine	50 µg/kg	Milk	

For other tissues except milk, the inclusion of novobiocin in Annex II to Council Regulation (EEC) No. 2377/90 is recommended in accordance with the following table:

Pharmacologically active Substance (s)	Animal species	Other provisions
Novobiocin	Bovine	For intramammary use only and for all tissues except milk

Based on this MRL value, the daily intake will represent the 100% of the microbiological ADI.