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

EMEA/MRL/466/98-FINAL
July 1998

COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

NICOBOXIL

SUMMARY REPORT

1. Nicoboxil (CAS 13912-80-6; synonym: 3-pyridinecarboxylic acid, 2-butoxyethyl ester) is an ester of nicotinic acid (niacin) and 2-butoxyethanol. In veterinary medicine nicoboxil is used in combination together with nonivamide as rubefacient in liniments for topical treatment of locomotor disorders, oedemas or haematomas. This application relates to the topical use of nicoboxil in horses. The intended daily dose is approximately 20 mg/horse corresponding to about 40 to 60 µg/kg bw as single dose. The treatment can be repeated over several days.

   A combination product of nicoboxil and nonivamide is also used as local rubefacient in human medicine.

2. Nicoboxil was reported to act on innervation of blood vessels of the skin leading to local reactions like vascular dilatation and hyperaemia. Data on other pharmacodynamic effects were not available. The ester hydrolysis product nicotinic acid is a vitamin of the B complex required by the body for the formation of the coenzymes NAD and NADP. The daily requirement of nicotinic acid as a supplement in humans is in the range of 10 to 20 mg. Nicotinic acid alone also leads to vascular dilatation and hyperaemia. The second hydrolysis product, the glycol ether 2-butoxyethanol, is a widely used industrial solvent and is also contained in household products. It has skin irritating properties and leads to haemolysis and decreased red blood cell counts and haematocrit in several rodent species. Humans and guinea pigs are reported to be less sensitive.

3. Specific investigations on absorption of dermally applied nicoboxil in laboratory animals or target species were not available. Published data for related nicotinate esters indicated however, that members of this class of compounds are in principle able to penetrate skin. There was some evidence from *in vitro* data that any absorbed nicoboxil can be expected to be hydrolysed to nicotinic acid and 2-butoxyethanol in blood plasma. *In vitro* the hydrolysis was reported to be catalysed by an esterase-like activity of serum albumin and by plasma esterases. The half-life of ester hydrolysis was found to be very short in the presence of human serum albumin (less than 15 minutes, 50 µM). Following ester cleavage, the nicotinic acid moiety, as a part of the vitamin B complex, may be assumed to enter the endogenous metabolic pool. Published pharmacokinetic data for the 2-butoxyethanol moiety suggested that this substance is mainly excreted via urine and exhaled air, predominantly in metabolised form. In rats given orally ^{14}C-2-butoxyethanol (absorbed dose of 11.8 to 171.1 mg/kg bw) within 72 hours after treatment, about 70% of the dose was recovered in urine in form of the main metabolites 2-butoxyacetic acid (50 to 60%) and ethylene glycol (10%). About 8 to 10% of the dose was exhaled as CO_2 and less than 5% as unmetabolised glycol ether. Urinary elimination in form of the metabolite 2-butoxyacetic acid was also reported for the dermal and inhalation route of administration of 2-butoxyethanol in rats and man.

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4. Nicoboxil is of low acute toxicity. The oral LD_{50} in rats was reported to be 4300 mg/kg bw and dermal LD_{50} in rabbits was 4640 mg/kg bw. Toxic symptoms were hyperaemia followed by paleness and laboured respiration, ataxia, sprawling of limbs and depression. In combination with nonivamide, the acute dermal toxicity was found to be increased. Toxic signs (depression, laboured respiration, diarrhoea) were seen at doses as low as 200 mg/kg bw nicoboxil plus 32 mg nonivamide/kg bw.

5. Repeated dose toxicity was studied in rabbits for a combination of nicoboxil and nonivamide using dermal administration of a cream aerosol at dose levels of 0.25 and 1 mg nicoboxil/kg bw and 0.38 and 1.5 mg nonivamide/kg bw (3 animals/sex/dose). The dose was administered over 3 weeks for about 8 hours daily on 6 days a week. Observed effects were a dose dependent decrease in leukocyte cell count in treated animals and lowered red blood cell parameters in one female of the highest dose group. The toxicological significance of this effect was not clear. Besides slight skin irritation in the highest group no other significant changes were noted.

   Oral repeated dose toxicity studies were not provided.

   2-Butoxyethanol and its main metabolite butoxyacetic acid were reported to be haemolytic in rats, rabbits and baboons, but much less so in pigs, guinea pigs and humans. However, in a study on workers exposed at the working place to concentrations of 0.59 ppm (2.91 mg 2-butoxyethanol/m^3) in the air a significant decrease in haematocrit values was reported.

6. Reproduction toxicity or teratogenicity studies for nicoboxil or the hydrolysation products were not provided. Such studies are considered not to be necessary for the vitamin moiety niacin. Some data described in published literature indicated that the glycol ether 2-butoxyethanol may be embryo- and/or foetotoxic in mice, rats and rabbits at maternal toxic doses but it was not found to be teratogenic at the doses tested. 2-Butoxyethanol given orally to male and female mice over a 98-day cohabitation period (700, 1300, and 2100 mg/kg bw) was found to elicit female reproductive performance disturbances and embryo/foetotoxic effects at maternotoxic doses greater than 700 mg/kg bw only. Given to rats on day 7 to 16 of gestation 4 times daily at a dermal dose of 116.2 mg (464.8 mg daily total), 2-butoxyethanol was reported to show no embryo/foetotoxic or teratogenic effects while a dose of 318.6 mg (1274.4 mg daily total) led to death in most of the dams (10 out of 11). Exposure of rats and rabbits by inhalation on gestational days (from day 6 to 15 for rats and 6 to 18 for rabbits) at concentrations of 25, 50, 100 or 200 ppm resulted in maternal toxicity and embryo/foeto toxicity at 200 or 100 ppm. At 50 or 25 ppm, no maternal, embryo or foetal toxicity was observed. In rabbits, only the highest dose of 200 ppm was found to result in maternal toxicity and reduced numbers of total and viable implantations/litter but not in foetotoxicity. No treatment-related malformations were reported for rats or rabbits in any exposure group.

7. Mutagenicity studies for nicoboxil, nicotinic acid or 2-butoxyethanol were not provided. As reported in published literature, 2-butoxyethanol and the metabolites 2-butoxyacetaldehyde and 2-butoxy-acetic acid have been tested for genotoxic activity in a range of *in vitro* and *in vivo* assays. This included gene mutation in bacteria (*Salmonella*) and mammalian cells (e.g. CHO/HPRT) as well as tests for chromosomal aberrations and sister chromatid exchange, *in vivo* mouse or rat bone marrow micronucleus assays and others. The available data were evaluated in a recent review article. Though some of the test results were partly positive or considered equivocal, the overall conclusion was that 2-butoxyethanol and the major metabolite 2-butoxyacetic acid do not have any significant genotoxic properties or possess any chemical structures alerting for genotoxicity.

8. No studies on carcinogenic properties of nicoboxil or its ester cleavage products were provided but were not considered necessary in view of the conclusion on mutagenicity.

9. No specific studies on immunotoxicity were provided.

10. No data on antimicrobial activity on human gut flora were available but these are not considered necessary for the type of compound.

11. No adverse side effects were reported in humans after therapeutic use of the combination of nicoboxil/nonivamide. In a study using dermal application of nicoboxil/nonivamide in over 1000 patients allergic reaction were not observed.

    The hydrolysis product 2-butoxyethanol has shown skin irritating properties. No information on allergic responses to this compound was available.

12. Residue studies in horses were not available, but are not considered necessary as pharmacokinetic data indicate rapid metabolism and elimination of nicoboxil.

Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances into Annex II of Council Regulation (EEC) No 2377/90 and in particular that:

- nicoboxil is used only for topical treatment of individual animals at very low doses,
- animals are unlikely to be sent for slaughter during or immediately after treatment,
- nicoboxil is expected to be readily hydrolysed to the vitamin moiety nicotinic acid and the glycol ether 2-butoxyethanol, which is quickly metabolised and eliminated,
- nicoboxil is of low oral acute toxicity;

the Committee concludes that there is no need to establish MRLs for nicoboxil and recommends its inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Nicoboxil	Equidae	For topical use only