The European Agency for the Evaluation of Medicinal Products
Veterinary Medicines and inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BRONOPOL

SUMMARY REPORT (1)

1. Bronopol (2-bromo-2-nitropropane-1,3-diol) is an antimicrobial preservative, which is used in shampoos, cosmetics, in food-contact materials and in topical pharmaceutical preparations intended for human use. It has now been proposed as a bath treatment for control of fungal infections in farmed Salmonid eggs, at a dose rate of 50 mg bronopol per litre of water for 30 minutes. There may be up to 8 treatments each of 30 minutes during a typical incubation.

2. Bronopol was reported to be active against a wide range of bacteria but to be less active against moulds and yeasts. The precise antimicrobial mode of action of bronopol is not known. It is believed that bronopol causes inhibition of thiol-containing dehydrogenase enzymes that are membrane bound, resulting in cell leakage and eventual collapse. There were no data on the secondary pharmacological effects of bronopol in laboratory animals. The repeated-dose toxicity studies were carried out during the early 1970s when parameters such as blood pressure and electrocardiogram were not routinely monitored. Consequently it was possible that some effects of the substance may have been missed.

3. After administration of a single oral dose of 1 mg/kg bw ^{14}C-bronopol to rats and Beagle dogs, peak plasma concentrations in the range of 0.5 to 0.61 and of 0.92 to 1.4 µg-equivalents/ml were observed in rats and dogs respectively, 0.5 to 2 hours after dosing. The bioavailability in rats after a single oral dose of 10 mg/kg bw or 50 mg/kg bw was estimated to be at least 75% in both cases. After single or repeated oral dosing to rats, bronopol-related residues were widely distributed to the tissues; there was no evidence of accumulation and excretion was rapid. Following a single oral dose of 10 mg/kg bw ^{14}C-bronopol to rats, over 60% of the administered dose was recovered from the urine during the first 12 hours after dosing and 1.3 to 7.4% from the faeces. Total faecal excretion for the first 168 hours after dosing accounted for approximately 10% of the administered dose. In the first 48 hours after dosing 4.1% and 1.8% of the dose was recovered in males and females respectively, from expired carbon dioxide.

4. Unmetabolised bronopol was not detected in the plasma or urine of treated rats at any time point. The main component in rat urine collected up to 24 hours after oral dosing with 10 or 50 mg/kg bw ^{14}C-bronopol was 2-nitropropane-1,3-diol which accounted for around 50% of the radioactivity present. Other metabolites could not be identified due to technical problems. In an *in vitro* experiment, samples of ^{14}C-bronopol added to samples of rat, dog and rabbit plasma were completely degraded within 5 minutes yielding 2-nitropropane-1,3-diol. In a special study designed to investigate the fate of the bromine moiety, rats were given a single oral dose of 1 or 50 mg/kg bw of bronopol and the resulting urine was analysed for bromide ion. It was claimed that the 1 mg/kg bw dose resulted in bromide ion concentrations which were not above the endogenous level and that around 17% of the 50 mg/kg bw dose was excreted as bromide over the period 0 to 120 hours.

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5. At pH 7 and 9, the rate of hydrolysis of bronopol was rapid but showed a distinct concentration dependence with the rate of hydrolysis increasing as concentrations were reduced. At bronopol concentrations in the range 0.01 to 0.1%, the hydrolysis half-life was less than one day at 25ºC. At pH 4, bronopol was intrinsically more stable but the results indicated a steady hydrolysis with time and a similar though less marked concentration effect. Four degradation pathways were identified; three of these proceeded via 2-bromo-2-nitroethanol, which was formed by the reversible loss of formaldehyde. The fourth pathway involved the irreversible conversion of bronopol into tris(hydroxymethyl)nitromethane.

6. Bronopol was of moderate acute toxicity. The acute oral LD_{50} values ranged from 180 to 400 mg/kg bw in rats and 250 to 500 mg/kg bw in mice. In rats, signs of toxicity were usually observed within 30 minutes of oral dosing and included sedation, salivation, wheezing, gasping or laboured breathing, nasal exudate, cyanosis and ataxia. Gross pathological examination of rats, which died revealed gastrointestinal irritation, small spleens and enlarged, dark-coloured adrenals. The acute LD_{50} after percutaneous administration to rats was reported to be greater than 1600 mg/kg bw. In rats, the LC_{50} value following a 6-hour inhalational exposure was 5 mg/l of air.

7. Groups of 20/sex/dose Charles River CD rats were given daily oral gavage doses of 0 (distilled water), 20, 80 or 160 mg/kg bw per day of bronopol for 13 weeks. All the rats given 160 mg/kg bw died or were euthanased by day 9 of the study. Seven males and nine females in the 80 mg/kg bw group also died. Overt signs of toxicity were observed at 80 and 160 mg/kg bw and in one rat given 20 mg/kg bw and included respiratory distress and abdominal distension. Body weight gain and food consumption were adversely affected at 80 and 160 mg/kg bw. Pathological changes in the rats, which died included gaseous or fluid distension in the gastrointestinal tract, distension of the lung lobes and regressive changes in the thyroid. Terminal histopathology revealed renal changes (dilated tubules containing eosinophilic material) in 2 rats given 20 mg/kg bw and 2 given 80 mg/kg bw. The study was poorly conducted with a limited range of clinical chemistry and histopathological investigations and no analysis of the dosing solutions. No NOEL was established.

8. Groups of 3/sex/dose Beagle dogs were given daily oral gavage doses of 0 (distilled water), 4, 8 or 20 mg/kg bw per day of bronopol for 13 weeks. For the first 6 weeks, the dogs given 20 mg/kg bw vomited around 30 minutes after dosing. Thereafter the dogs were fed 2 hours before dosing and vomitus was observed in only one dog on one occasion. After 12 weeks of dosing, white blood cell counts were significantly reduced in the groups given 8 and 20 mg/kg bw. At termination, mean absolute and relative spleen weights and mean relative liver weights were significantly increased in the 20 mg/kg bw group but there were no corresponding pathological changes. The study was poorly conducted with a limited range of clinical chemistry investigations and no analysis of the dosing solutions. The NOEL, based on reduced white blood cell counts, was 4 mg/kg bw per day.

9. Target species tolerance studies were provided for rainbow trout eggs. Mortalities showed marked differences between sites and among different batches of eggs at the same site. However, overall mortalities at hatching in the groups treated with bronopol were comparable with those treated with malachite green and were significantly lower than the untreated controls.

10. In a single-generation study, groups of 11 male and 22 female Charles River CD rats were given daily oral doses of 20 or 40 mg/kg bw per day of bronopol, dissolved in distilled water. The males were treated for 63 days and the females for 14 days prior to mating. A further group of 11 males and 22 females was left untreated. On day 13 of gestation, 10 dams/dose were necropsied; the remainder were allowed to litter down naturally and rear their young to weaning. There were no substance-related effects on fertility or mating performance, pregnancy rate, length of gestation, corpora lutea, numbers of implantations, resorptions or viable foetuses. The study was pre-GLP and there was no analysis of the dosing solutions.

11. In a GLP-compliant study, groups of 24 mated female Sprague-Dawley rats were given daily oral doses of 0, 10, 28 or 80 mg/kg bw per day of bronopol from days 6-15 of gestation. The solvent vehicle was purified water acidified to pH 4 with 1.0 M hydrochloric acid. Maternal toxicity (significantly reduced body weight gain over days 6-7 of gestation) was observed at 80 mg/kg bw. There were no effects on the numbers of resorptions, live foetuses, foetal sex ratio or foetal weight. There was no evidence of teratogenicity at any dose level. In the 80 mg/kg bw group, the incidence of foetuses with incomplete ossification of the sacral neural arcs was significantly reduced compared with the controls. However, it was considered that this was a fortuitous finding unrelated to treatment. The incidence in the 80 mg/kg bw group was within the historical control range whereas the incidence in the controls was higher than the background range. The NOELs were 28 mg/kg bw for maternal toxicity and greater than 80 mg/kg bw for teratogenicity and foetotoxicity.

12. In a GLP-compliant study, groups of 18 to 20 mated female New Zealand White rabbits were given daily oral doses of 0, 5, 20, 40 or 80 mg/kg bw per day of bronopol from days 7 to 19 of gestation. Again the solvent vehicle was purified water acidified to pH 4. Maternal toxicity was observed at 80 mg/kg bw with reduced food consumption, body weight loss up to day 11 and reduced quantity and size of faecal pellets. In the 80 mg/kg bw group, mean foetal weights were significantly reduced and the incidence of runts was significantly increased. The incidence of malformations in the 80 mg/kg bw group was not significantly different from the controls but was higher than the historical control range. The incidence of foetuses with skeletal variations was significantly higher in the 80 mg/kg bw group due to higher incidences of foetuses with non or retarded ossification of the pubic bones and other bones of the skull, vertebrae, sternum and limbs. The NOEL for maternal toxicity, foetotoxicity and teratogenicity was 40 mg/kg bw per day.

13. Dose levels of 0 (distilled water), 20 and 40 mg/kg bw per day were given by oral gavage in a study on peri- and post-natal development. Groups of 20 mated female rats were treated from day 15 of gestation, through lactation, up to day 21 post-partum. One dam given 20 mg/kg bw and one given 40 mg/kg bw died; necropsy revealed gastrointestinal bleeding suggesting that administration of the test substance may have been a contributory factor in the deaths. From day 4 post-partum, pup mortality was significantly increased in both groups compared with the controls and pup weights were slightly but not significantly reduced. No NOEL was established. The study was pre-GLP and there was no analysis of the dosing solutions.

14. Negative results were obtained in *in vitro* assays for gene mutation in *Salmonella typhimurium* G46, TA1535, TA1536, TA1537, TA1538, TA98 and TA100, in E. coli WP2, UvrA, CM561 and CM611 and in an *in vitro* assay for gene mutation in Chinese Hamster V79 cells. In a host-mediated assay, groups of female mice administered daily oral doses of 0, 12.5, 25 or 50 mg/kg bw per day for 6 days with the final dose given concurrently with the indicator organism *Salmonella typhimurium* TA1530; negative results were obtained. In an *in vitro* cytogenetics assay in human lymphocytes, there was a reproducible significant increase in the percentage of cells with aberrations at the top concentration of 30 µg/ml in the absence of metabolic activation. A negative result was obtained in the presence of metabolic activation but this part of the assay was not repeated. It was shown that bronopol degraded to the known clastogen formaldehyde under the culture conditions employed and produced a dose-related increase in chromosomal aberrations in the same assay. Therefore, the positive result in the *in vitro* cytogenetics assay with bronopol may have been due to the formaldehyde degradation product. A negative result was obtained in an *in vivo* micronucleus test in which groups of mice were given an oral dose of 80 or 160 mg/kg bw bronopol and their bone marrow was harvested 24, 48 or 72 hours later. Negative results were obtained in a dominant lethal assay in which groups of male mice were administered daily oral doses of 20 or 60 mg/kg bw per day for 6 days or a single intraperitoneal injection of 10 mg/kg bw bronopol prior to mating with untreated females. It was concluded that bronopol was not genotoxic.

15. A carcinogenicity study was carried out in which groups of 52/sex/dose Carworth CFLP mice were treated topically with 0, 0.2% or 0.5% solution of bronopol (in 90% acetone/water). The area to be treated was shaved and the test substance was applied at a rate of 0.3 ml per mouse on 3 days per week, for 80 weeks. The doses corresponded to 0, 4.9/6.5 and 12.7/16.5 mg/kg bw per day in males/females respectively. Slight hair loss was observed at the periphery of the shaved area during the first 3 weeks. Mean body weight gain was significantly reduced in male mice given the top dose of 0.5% bronopol but food consumption was unaffected by treatment. The study was carried out during the early 1970s and only a limited number of tissues were examined microscopically at that time. About 10 years later, the remainder of the tissues from the groups were examined. Combining the 2 histology reports revealed a previously unreported treatment-related incidence of papillomas on the treated skin site; papillomas were found in one male and 3 females treated with the top dose of 0.5% bronopol but none on the other groups; in females, the incidence of papillomas showed a statistically significant positive trend ($p < 0.033$). There was also an increase in the incidence of nodular hyperplasia of the liver in males given 0.5% but the increase was not statistically significant. It was considered that the papillomas observed at 0.5% (equivalent to 12.7/16.5 mg/kg bw per day in males/females) were a consequence of the non-genotoxic local irritant effect of bronopol.

16. In a combined chronic toxicity and carcinogenicity study, groups of 45/sex/dose Sprague-Dawley derived rats were given bronopol in the drinking water at concentrations designed to provide 0, 10, 40 or 160 mg/kg bw per day for up to 104 weeks. Satellite groups of 15/sex/dose rats were used for blood sampling and were not included in the carcinogenicity assessment. Mortality was significantly increased in the 160 mg/kg bw group with only 20% of males and 38% of females from the main groups surviving to termination. Body weight gain and food consumption were significantly reduced in the 40 and 160 mg/kg bw groups. There was a significant dose-related reduction in water intake in all treated groups. There were no consistent dose-related trends in haematology or clinical chemistry values. Urinalysis which was monitored only in the control and 160 mg/kg bw group showed that rats given 160 mg/kg bw excreted a smaller volume of urine. The study was carried out during the early 1970s and only a limited number of tissues were examined microscopically at that time. About 10 years later, the remainder of the tissues from the groups were examined. Several papillomas of the fore-stomach were found in treated males but the incidence was not statistically-significant. A range of non-neoplastic lesions were also observed and appeared to be associated with the irritant effect of bronopol: ulceration and hyperkeratosis of the forestomach in the 160 mg/kg bw groups, an increased incidence of glandular dilatation of the secretory stomach in the 40 mg/kg bw group, an increased incidence of hyperkeratosis of the oesophageal epithelium and/or ulceration in the 40 and 160 mg/kg bw groups, an increased incidence of lesions of the salivary gland (squamous metaplasia, fibrosis, ductal dilatation/cysts) in the 40 and 160 mg/kg bw groups, an increased incidence of glandular dilatation of the trachea in the 40 and 160 mg/kg bw groups and ulceration of the tongue in the groups given 40 and 160 mg/kg bw. There was no analysis of the dosing solutions used in the study. Subsequent work on the stability of bronopol under the conditions employed suggested that the actual doses were 7, 32 and 142 mg/kg bw rather than the intended 10, 40 and 160 mg/kg bw. The study was inadequate as an assessment of carcinogenicity due to the small group sizes and poor survival. No overall NOEL was established due to the reduced water intake observed at the lowest dose level.

17. Sensitisation studies were carried out in guinea pigs using a version of the Magnussen & Kligman test. In these studies, the sensitisation potential of bronopol was low. Negative results were obtained on challenge with formalin. This low potential was confirmed in tests in human volunteers; no evidence of sensitisation was seen in one study in 120 people. Seven subjects with allergic skin reactions were observed in another study in 127 patients.

18. *In vitro* MIC data were provided for six strains (3 genera) of Gram-positive bacteria and 15 strains (7 genera) of Gram-negative bacteria. The species tested included several which were representative of the normal human gut flora. MIC values were generally in the range 12.5 to 25 µg/ml indicating that the bacteria were relatively insensitive to Bronopol. No studies were provided on effects of bronopol on the food industrial processes were carried out; it was considered that such data were not necessary bearing in mind the proposed use of the substance.

19. Bronopol is widely used in products intended for topical application to humans. The extent of absorption varied between individuals with most of the dose remaining at the site of application. Up to 5% of a topically applied dose of ^{14}C-bronopol was excreted in urine up to 48 hours after application but no residues were detectable in faeces.

20. A toxicological ADI of 20 µg/kg bw (1200 µg/person) may be calculated for bronopol by applying a safety factor of 200 to the NOEL of 4 mg/kg bw per day, which was established in the 13-week repeated-dose toxicity study in dogs. A safety factor of 200 was used to compensate for the limited range of clinical chemistry investigations carried out in this study and the lack of analysis of the dosing solutions.

21. No metabolism, pharmacokinetic or residues depletion data were provided for bronopol in the target species. In mammals, bronopol was rapidly metabolised and excreted; residues of unmetabolised bronopol were undetectable in plasma or urine at any time point. Bronopol was also shown to be quickly hydrolysed in aqueous solution. Because the growth of salmonids up to table size takes a minimum of 12 months from hatching, it was concluded that residues of bronopol used to treat the eggs would have long since disappeared. The absence of residue data was therefore justified.

Conclusions and recommendation

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

- bronopol was rapidly metabolised and excreted in mammals,
- bronopol is to be administered only on farmed fertilised salmonid eggs,
- the use of bronopol on farmed fertilised salmonid eggs should not result in detectable residues in adult fish;

the Committee considers that there is no need to establish an MRL for bronopol 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
Bronopol	Salmonidae	For use only on farmed fertilised eggs