The European Agency for the Evaluation of Medicinal Products

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

BITUMINOSULFONATES, AMMONIUM AND SODIUM SALTS

SUMMARY REPORT

1. The ammonium salt of dark bituminosulfonate (CAS No 8029-68-3) is prepared by destructive distillation of bituminous schists or shale, together with ammonium sulphate and water. The sodium salt of dark bituminosulfonate is obtained by the destructive distillation of certain bituminous schists, sulphonation of the distillate and neutralisation of the product with sodium hydroxide. The ammonium salt of light bituminosulfonate is produced from the light distillate fraction of shale oil and the sodium salt of light bituminosulfonate (CAS No 12542-33-5) is produced from the light distillate fraction of shale oil. Monographs for light and dark bituminosulfonates are included in the German and United Kingdom pharmacopoeias, these specify limits for polycyclic aromatic hydrocarbons (as benzo[*a*]pyrene) of 10 and 100 mg/kg, respectively.

   In veterinary medicine, bituminosulfonates are applied topically to the skin of horses, pigs, cattle, rabbits and other animals. They may be applied undiluted or in ointments at a concentration of 20 to 50%, for the treatment of skin diseases.

   The bituminosulfonates are used in human medicine. The products include an oral preparation containing sodium bituminosulfonate which is intended for administration at a dose of 1200 mg per day, for 14 days, followed by a further 7 days on half-dose; it is used for the treatment of diseases such as acne, seborrhoea, rosacea and furunculosis. A wide range of topical preparations are also available, for the treatment of skin disorders such as eczema and psoriasis.

2. The bituminosulfonates have bacteriostatic properties. The action of bituminosulfonates in treatment of inflammatory skin disorders is based on inhibitory effects on cell migration, ATPase activity of epidermal Langerhans cells and release of chemotactic factors such as leukotriene B_4 (LTB_4). No pharmacological NOEL could be identified from the available data.

3. An oral study in the rat using ^{35}S-labelled sodium salt of dark bituminosulfonate (single doses of 20 or 40.4 mg/kg bw or 5 doses of 17.4 or 17.8 mg/kg bw) indicated relatively rapid absorption (bioavailability is larger than 33%), and elimination with little evidence of bioaccumulation. No studies have been conducted on the metabolism of dark or light bituminosulfonates. Analogies have been made to other thiophene compounds, suggesting a major metabolic route may be direct conjugation with glycine by a mechanism similar to the formation of hippuric acid. Minor routes involving the synthesis of mercapturic acids and sulfonation or glucuronidation of alkyl side chains have also been suggested. No data were provided on the pharmacokinetics of light bituminosulfonates.

   Co-administration with ammoniumbituminosulfonates was reported to enhance the percutaneous absorption of topically applied corticosteroids, chloramphenicol and heparin.

4. Oral LD_{50} values for a sodium salt of dark bituminosulfonate of 6750 mg/kg bw (males) and 6800 mg/kg bw (females) were determined in the rat, the lowest toxic dose was 3180 mg/kg bw. Using a sodium salt of light bituminosulfonate, no deaths occurred, slight sedation was observed at 7900 mg/kg bw and sedation and ataxia at 10 000 mg/kg bw.

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5. A non-GLP 6 month dietary study was conducted in rats using a sodium salt of dark bituminosulfonate (0, 330, 100 or 3000 mg/kg bw, 20 animals per sex per group). No deaths occurred. Reduced bodyweight gain and food consumption were observed at 3000 mg/kg bw. Significant organ weight reductions were seen at 3000 mg/kg bw, affecting liver, spleen and thymus in males and liver, kidneys, thymus and pituitary in females. No treatment-related effects were reported at necropsy or histology. A NOEL of 1000 mg/kg bw was identified, based on the effects on bodyweight, food intake and organ weights at 3000 mg/kg bw.

   A non-GLP 6-month oral gavage study was conducted in Beagle dogs (3 animals per sex per group) using a sodium salt of dark bituminosulfonate (0, 330 or 990 mg/kg bw). No deaths occurred. Vomiting, hypersalivation, delayed and reduced food intake, and reduced bodyweight gain were observed at 990 mg/kg bw. Significant increases in serum alanine and aspartate aminotransferase levels were observed from week 18 at 990 mg/kg bw. Liver weight was significantly increased at 990 mg/kg bw. Hepatocyte hydropic degeneration and hypertrophy were observed in 2 out of 3 males and 2 out of 3 females at 990 mg/kg bw. A NOEL of 330 mg/kg bw was identified.

   No repeated dose toxicity studies were conducted using light bituminosulfonates.

6. No data on target species tolerance was provided.

7. No reproduction studies have been conducted on dark bituminosulfonates. A non-GLP one-generation reproduction study was conducted in Sprague-Dawley rats (20 animals per sex per group) using a sodium salt of light bituminosulfonates (0, 100, 500 or 2500 mg/kg bw by oral gavage) from 10 weeks before mating until completion of the study. No treatment-related clinical signs were observed, or effects on fertility, weight gain and food intake in the parent animals. No treatment-related effects on numbers of corpora lutea, implantations, live or dead foetuses or resorptions were observed in the dams sacrificed on day 13 of gestation. No treatment-related effects on numbers of pups, neonatal weights or weight gain, runts, stillbirths or malformations were observed. A NOEL of 2500 mg/kg bw was identified for effects on fertility.

8. A non-GLP teratology study was conducted in rats (20 pregnant females per group) using a sodium salt of dark bituminosulfonate (0, 300, 900 or 2700 mg/kg bw by oral gavage from days 6 to 15 of gestation). No treatment-related effects on maternal bodyweights, food consumption, clinical signs or gross necropsy findings were observed. No treatment-related effects on foetal weights malformations or variations were observed. There was a slight increase in post-implantation loss (13.3%), mean resorptions (1.7 ± 3.1), and 1 dead foetus at 2700 mg/kg bw, compared to controls (8.7%, 1.1 ± 1.5 and 0, respectively), largely attributable to one high-dose litter. A NOEL of 900 mg/kg bw was identified.

   A non-GLP teratology study was conducted in rats (20 pregnant females per group) using a sodium salt of light bituminosulfonate (0 or 1000 mg/kg bw from days 6 to 15 of gestation). No treatment-related effects on body weights, food consumption, clinical signs or gross necropsy findings were observed. Fertility and litter parameters were comparable in both treated and untreated groups. No runts, malformed or dead foetuses were observed in either group and incidence of variations was comparable.

   A non-GLP teratology study was conducted in New Zealand White rabbits (30 pregnant females per group) using a sodium salt of light bituminosulfonate (0, 100, 400 or 1600 mg/kg bw by oral gavage from days 6 to 18 of gestation). At 1600 mg/kg bw bodyweight gain and food intake were significantly reduced on days 18 and 29 of gestation, and clinical signs of ataxia, apathy and sedation were observed. Post-implantation loss and resorptions were significantly increased and number of viable foetuses and foetal weight were significantly decreased at 1600 mg/kg bw and there was a marginal increase in post-implantation loss at 400 mg/kg bw. A second non-GLP study was conducted in New Zealand White rabbits (30 pregnant females per group) using a sodium salt of light bituminosulfonate (0, 500, 1000 or 1500 mg/kg bw by oral gavage from days 6 to 18 of gestation). Maternal bodyweight gain and food intake was reduced at 1500 mg/kg bw, the reduction in bodyweight was statistically significant on day 18. Clinical signs of diarrhoea, ataxia, sedation and proneness were seen in this group. Non-significant decreases in bodyweight gain and food intake were observed at 1000 mg/kg bw. Post-implantation loss and resorptions were significantly increased and number of viable foetuses

and placental weight were significantly decreased at 1500 mg/kg bw. NOELs for maternal and foetotoxicity of 500 and 1000 mg/kg bw, respectively, were identified.

9. A *Salmonella*-microsomal assay was conducted using a sodium salt of dark bituminosulfonate. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were treated with concentrations of 3.16 to 10 000 µg/plate, both with and without metabolic activation. There was no evidence of mutagenicity at any concentration tested. A GLP-compliant *in vitro* point mutation assay (HPRT locus) study using Chinese hamster V79 lung fibroblasts was conducted using a sodium salt of dark bituminosulfonate. Cells were exposed to concentrations of 31.3 to 500 µl/ml for 24 hours, with metabolic activation, or 93.8 to 3 000 µl/ml for 2 hours with metabolic activation. No treatment-related effects on cloning efficiency, thioguanine resistant or mutation frequency were observed in the treated animals compared to controls. Cytotoxicity was observed at concentrations of 250 and 500 µl/ml (without metabolic activation) and 1500 and 3000 µl/ml (with metabolic activation). A bone marrow chromosome aberration study in Chinese hamsters was conducted using a sodium salt of dark bituminosulfonate. Groups of animals (5 per sex) received oral doses of 2500, 5000 or 10 000 mg/kg bw. Groups were sacrificed at 6, 24 and 48 hours after dosing, having previously received an intraperitoneal dose of 4 mg desacetylmethylcolchicine/kg bw. No cytotoxicity was observed at any of the doses used, but at 10 000 mg/kg bw 20% mortality occurred within 24 hours, and 40% mortality within 48 hours. No significant differences in chromosome/chromatid aberrations or mitotic indices were observed in the treated groups, compared to vehicle controls at any time point.

   A *Salmonella*-microsomal assay was conducted using light bituminosulfonates. *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 were treated with concentrations of 0.1 to 100 µg/plate, both with and without metabolic activation. Slight cytotoxicity in the form of disturbance of the background lawn was noted in most strains at 100 µg/ml and occasionally at 31.6 µg/ml in the series with metabolic activation and occasionally at 100 µg/ml with metabolic activation. There was no evidence of mutagenicity at any concentration tested. A rat bone marrow chromosome aberration study was conducted using light bituminosulfonates. Groups of rats (5 per sex) received oral doses of approximately 1200 mg/kg bw. Groups were sacrificed at 6, 24 and 48 hours after dosing, having previously received an intraperitoneal dose of 4 mg desacetylmethylcolchicine/kg bw. No significant differences in chromosome/chromatid aberrations or mitotic indices were observed in the treated groups, compared to vehicle controls at any time point.

   All mutagenicity studies were conducted in accordance with current guidelines and complied to GLP. All three *in vitro* and the two *in vivo* studies gave negative results. The *in vivo* studies could, possibly, be criticised for although both used doses up to, or exceeding the maximum tolerated dose, there was no evidence of cytotoxicity in the bone marrow, which may cast doubt on whether an adequate concentration of the test material reached the target tissue.

10. A non-GLP life-time feeding study was conducted in Wistar rats, using a sodium salt of dark bituminosulfonate. A group of 19 male and 20 female rats were fed a diet containing the equivalent of 5 mg/kg bw from 100 days old until their natural death. There was a treatment-related decrease in survival in the dosed females with 50% survival at 24 months and a mean survival of 743 days, compared to 68% and 778 days in controls, male survival was slightly affected. There was a slight increase in overall tumour rates in the treated animals (males 47%, females 40%, compared to 34% and 37% in controls), and a relatively high incidence (but not statistically significant) of malignant tumours in treated females (30% compared to 18% in controls). The commonest site for malignant tumours in the control females was the haemopoietic system with none in the gastrointestinal tract. In the treated females malignant tumours occurred most often in the gastrointestinal tract (3 tumours (38%) in 2 animals (10%)), with only one in the haemopoietic system. A malignant tumour was also observed in the gastrointestinal tract of one treated male. No malignant gastrointestinal tumours were observed in controls, only one benign tumour in one female.

A non-GLP 24-month dietary carcinogenicity study was conducted in rats (40 animals per sex per group) using a sodium salt of light bituminosulfonate (0, 330, 1000 or 3000 mg/kg bw for 24 months). No treatment-related clinical effects were reported. There was no evidence of treatment-related effects on mortality, with slight decreases in the low (8.8%) and high (11.3%) dose groups and a slight increase (16.3%) in the mid dose, compared to controls (13.8%). Bodyweight gain and food intake were significantly reduced in the 3000 mg/kg bw group, with significant decreases in spleen and heart weight in this group. Tumour incidence rates were lower in treated animals (low 12.5%, mid 15.0%, high 8.8%), compared to controls (17.5%).

A non-GLP 21-month dietary carcinogenicity study was conducted in NMRI mice (40 per sex per group) using a sodium salt of light bituminosulfonate (0, 330, 1000 or 3000 mg/kg bw for 21 months). Only a summary of the study was available indicating no treatment-related effects on overall tumour and mortality rates.

In a non-GLP 24-month study, twice weekly dermal applications of 50 mg bituminosulfonate to the intrascapular skin of mice did not produce any skin tumours.

None of these carcinogenicity studies were conducted to current standards or to GLP. The rat study on light bituminosulfonates appeared to be adequately conducted and showed no indication of tumourigenic effects up to the maximum tolerated dose of 3000 mg/kg bw. The rat study on dark bituminosulfonate was poorly conducted. In the only treatment group, there was evidence of decreased survival in females. The treated females also showed a higher incidence of malignant tumours and there was evidence of differences in tumour localisation, with malignancies commonly occurring in haemopoietic tissue in controls, but those in treated animals most frequent in the gastro-intestinal tract. The increased tumour incidence in treated animals was not statistically significant. The bituminosulfonates contain impurities including polycyclic aromatic hydrocarbons known to be carcinogens. The gastrointestinal tumours observed in treated animals were considered to be attributed to the polycyclic aromatic hydrocarbons content of the material used in the study. There have been significant reductions in the polycyclic aromatic hydrocarbons content of bituminosulfonates since this study was conducted, with mean concentrations of polycyclic aromatic hydrocarbons (as benzo[*a*]pyrene) in representative batches of dark bituminosulfonates falling from 78 µg/kg in 1994 to 11 µg/kg in 1996 to 1998. The results of this study are therefore not considered to be relevant to the material currently manufactured.

11. No specific studies on immunotoxicity were conducted. Both light and dark bituminosulfonates exhibit dermal and ocular irritancy. Analogy with coal tars has suggested the possibility of phototoxicity and photosensitisation. However, laboratory studies in the guinea pig and volunteer studies in humans using a sodium salt of light bituminosulfonate showed no evidence of photosensitisation or phototoxicity.

12. No data on the effects of bituminosulfonates on microorganisms was provided. Bituminosulfonates have antiseptic and disinfectant properties. However, given the topical route of application and low rate dermal absorption, residues in food tissues are unlikely to present a risk to the human gut microflora, or dairy starter organisms.

13. The reported adverse effects following oral administration in humans were limited to gastrointestinal disturbances. The main adverse effect following topical use was skin irritation. A few cases of hypersensitivity have also been reported.

14. No pharmacological NOELs were identified for the bituminosulfonates. However, the toxicological effects observed were considered to be related to small amounts of polycyclic aromatic hydrocarbons impurities, thus it was not considered necessary to establish a pharmacological ADI. Acute oral studies indicated marked differences between dark and light compounds with LD_{50} values of 6800 mg/kg bw and more than 10 000 mg/kg bw, respectively. Repeated dose studies in the rat and dog using dark bituminosulfonates gave NOELs of 1000 and 330 mg/kg bw, respectively. No repeated dose studies were conducted using light bituminosulfonates. There was no evidence of teratogenicity for either light or dark bituminosulfonates. *In vitro* and *in vivo* mutagenicity studies were negative,

although there was no evidence of cytotoxicity in the target tissue in the *in vivo* studies. No evidence of tumourigenicity were observed with light bituminosulfonate at doses of up to 3000 mg/kg bw.

A toxicological ADI was established for light and dark bituminosulfonates, using the NOEL of 330 mg/kg bw from the 6-month oral dog study and using a safety factor of 200 to take into account the deficiencies of the study, giving a value of 1.65 mg/kg bw (99 mg/person/day).

15. No residues depletion studies were carried out with the bituminosulfonates. A pharmacokinetic study was conducted using 12 male mini pigs weighing 28.6 ± 6.8 kg. Dermal administration of ^{35}S-labelled light ammonium bituminosulfonate (approximately 324 mg/kg bw) to 120 cm^2 for 24 hours resulted in a C_{max} of 186 ng/g blood within a t_{max} of 7 to 12 hours. Dermal absorption accounted for 1 to 3% of the administered dose. Eighty-eight percent of the absorbed dose was excreted within 240 hours. The highest tissue residue concentrations were detected in the application site skin and superficial fat averaging 1000 µg ^{35}S/kg. All other tissue samples assessed contained sulphur concentrations of between 8.2 µg/kg (peri-renal fat) and 100.4 µg/kg (liver). Assuming ingestion of the standard porcine food basket including the application site, from a treated animal given a nil withdrawal period, a 'worst case' calculation for consumer intake would be 2362 µg/day. This intake would be less than 2.4% of the ADI.

16. No data are available for excretion or residues in milk and therefore use of bituminosulfonates in animals producing milk intended for human consumption cannot be permitted.

Conclusions and recommendations

Having considered that:

- a toxicological ADI of 1.65 mg/kg bw was established,
- the product is intended for topical application,
- dermal absorption was only 1 to 3% after a 24 hour application in the pig,
- elimination was rapid after oral administration with little evidence of bioaccumulation,
- bituminosulfonates are used in individual animals and for infrequent and non-regular treatment,
- animals are unlikely to be sent for slaughter immediately after treatment,
- the worst case estimate of consumer intake at a zero withdrawal period would be less than 2.4% of the ADI;

the Committee considers that there is no need to establish an MRL for bituminosulfonates, ammonium and sodium salts and recommends their inclusion in Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Target species	Other provisions
Bituminosulfonates, ammonium and sodium salts	All mammalian food producing species	For topical use only. Not for use in animals from which milk is produced for human consumption.