European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR MEDICINAL PRODUCTS FOR VETERINARY USE

LINEAR ALKYL BENZENE SULPHONIC ACIDS
(Extension to sheep)

SUMMARY REPORT (2)

1. Linear alkyl benzene sulphonic acids are anionic surfactants that are used for post-milking teat dipping or spraying for dairy cows. Linear alkyl benzene sulphonic acids are mixtures of benzene sulphonic acids containing linear alkyl chains of different lengths (C_9: less than 1%, C_{10}: 8 to 16%, C_{11}: 26 to 38%, C_{12}: 26 to 38%, C_{13}: 15 to 27% and longer than C_{13}: less than 2.5%). The amount of linear alkyl benzene sulphonic acid in the products is 2%. The average dose per teat is about 1 ml of the product, equal to 80 mg of linear alkyl benzene sulphonic acid per cow per milking.

   Linear alkyl benzene sulphonic acids are commonly used as cleaning agents (household and personal care products). Linear alkyl benzene sulphonic acids are included as surface active agent in Commission Decision 96/335/EC of 8 May 1996 establishing an inventory and a common nomenclature of ingredients employed in cosmetic products.

   Linear dodecyl benzene sulphonic acid, under the synonym sodium dodecyl benzene sulphonate, has been included in the food additive list of the US Food and Drug Administration as a surface active agent in commercial detergents used in washing fruits and vegetables or to assist in lye peeling these foods. The tolerance limit has been set on equal to or less than 0.2% in wash water.

   Linear alkyl benzene sulphonic acids are currently included in Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Linear alkyl benzene sulphonic acids with alkyl chain lengths ranging from C_9 to C_{13} , containing less than 2.5% of chains longer than C_{13}	Bovine	For topical use only

2. A request was submitted to the EMEA for the extension of the existing entry in Annex II of Council Regulation (EEC) No. 2377/90 to ovine species. Linear alkyl benzene sulphonic acids are intended to be used as wetting agents in formulations for control and treatment of footrot and footscald in sheep at a concentration of 0.2% w/w in the formulation and 0.02% in the prepared footbath. The feet of the animals are completely immersed for two to thirty minutes and the treatment is repeated at weekly intervals for a minimum of up to three weeks, and thereafter at one to two weeks during the period when the infection is likely to spread.

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The scientific justification for this extension was assessed in accordance with the Position Paper Regarding Availability of Veterinary Medicines - Extrapolation of MRLs (EMEA/CVMP/457/03-FINAL) and taking into account the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187-00-FINAL).

3. In setting the ADI in the original assessment of linear alkyl benzene sulphonic acids, the following data were considered:

4. Hydrophobic and hydrophilic groups of the molecule are both essential for action of surfactants in detergents. According to a published study on the *in vitro* germicidal activity of teat dips the linear alkyl benzene sulphonic acid-containing product (1.94%) was shown to be completely effective against suspensions of *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus agalactiae* containing 10^8 bacteria/ml each following a contact time of 2 minutes.

   According to a published review document, in *in vitro* studies, the 50% haemolytic concentration for linear alkyl benzene sulphonic acid was 9 mg/l and the 50% inhibitory concentration for prothrombin time was 0.05 mmol/l (16.3 mg/l). Linear alkyl benzene sulphonic acid influenced the thermal denaturation of proteins *in vitro* indicating protein-linear alkyl benzene sulphonic acid interaction.

5. Published reports of pharmacokinetic data were presented. In rats, ^{14}C-labelled alkyl benzene sulphonate was administered daily in the diet at a concentration of 1.4 mg/kg feed (dose per kg bw not given) to 12 male Wistar rats (120 to 140 g) for 5 weeks. Radioactivity was mostly excreted in faeces (52%) and in urine (29%) during the 5-week feeding period. After a single intraperitoneal administration of ^{14}C-labelled alkyl benzene sulphonate (384.7 µg/rat), 85% of the dose was excreted during the first 24 hours and 95% within 10 days follow-up period. The main elimination route was via urine (50% of radioactivity), while 35% was excreted into faeces. However, during days 2 to 10 the percentage of radioactivity excreted into faeces was higher than that excreted into urine. No parent compound could be detected in faeces or urine but radioactivity was found in polar metabolites which were not further characterised.

   In another study, ^{35}S-labelled linear alkyl benzene sulphonate was administered to male albino rats (Charles River strain, 150 to 200 g bodyweight) as a single per oral dose of 0.6, 1.2, 8 and 40 mg/rat (3 to 5 rats/group). During the 3-day follow-up period, 40 to 58% of radioactivity was excreted in urine and 39 to 56% in faeces. In faeces, the proportion of parent compound was 19% of total radioactivity. About 70% of linear alkyl benzene sulphonate was absorbed after oral administration. Two urine metabolites chemically similar to methyl 4-(4'-methylsulfophenyl)-pentanoate were identified and were found to be a mixture of sulfophenyl butanoic acids and sulfophenyl pentanoic acids. Decomposition of linear alkyl benzene sulphonate in rats was suggested to occur by ϖ-oxidation followed by catabolism through a β-oxidation mechanism.

   In vitro studies have not shown any transdermal penetration of ^{14}C-labelled linear alkyl benzene sulphonic acids through preparations of rat or human skin. In *in vivo* studies in rats, 0.2 ml of 3 mM ^{14}C linear alkyl benzene sulphonic acids (equivalent to 250 µg) was applied on 7.5 cm^2 area of skin. These studies revealed deposition of ^{14}C-labelled linear alkyl benzene sulphonic acids on the skin surface and in the upper regions of the hair follicles, however, no transdermal penetration of the substance could be detected after an exposure of 15 minutes.

6. The oral toxicity of linear alkyl benzene sulphonic acids is low. LD_{50} values for rats and mice range from 404 to 1525 mg/kg bw and 1575 to 1950 mg/kg bw, respectively. Diarrhoea was observed in both species and deaths occurred within 24 hours.

7. Repeated dose toxicity studies have been carried out using linear alkyl benzene sulphonic acids or their sodium salts containing alkyl chains of different lengths. Repeated dose toxicity has been documented in rats in 5 published articles, one of which was conducted (60 females and 60 males/group) using only 1 dose level (0 and 100 mg of linear alkyl benzene sulphonic acids (chain length varying between C_{10} to C_{14})/l drinking water for 100 weeks). No differences were seen between test and control groups. No NOEL can be established due to deficiencies in the study design.

Wistar rats (about 150 g, 10 per sex and group) received the test product (dishwashing detergent containing linear alkyl benzene sulphonic acids) was mixed into drinking water at concentrations corresponding to 0, 0.015, 0.075 and 0.375 ml linear alkyl benzene sulphonic acid/kg bw for 6 months. In the 3rd group the dose was increased after 9 weeks to 0.563 and again after 8 weeks to 0.75 ml/kg bw for 9 weeks. No differences were seen in haematological or urinary parameters between control and treated animals. Males showed decreased weight gain in the 3rd dose group, but the change was reversible once the treatment was stopped. Organ weights of the third group animals (5 per sex) killed immediately after the treatment were lower than those of the controls. Only control and the 3rd treatment groups were examined histologically. The animals in the 3rd treatment group had small petechial haemorrhages (kidney, myocardium, lungs) and mucosal necrotic foci in the gastrointestinal tract. They also had extensive atrophy in adrenal glands and some atrophy in thymus. It was not possible to determine a dose-relationship as tissues from the intermediate doses were not examined. No NOEL can be established from the study due to limited data available.

Albino rats (FDRL strain, 15 animals per sex and group) received linear alkyl benzene sulphonic acid in feed at 0, 50 and 250 mg/kg bw for 12 weeks. Growth responses and food intake, and haematological and urinary parameters showed no abnormalities. Histological findings revealed no abnormalities in lower dose group compared with control. Females in higher dose group had higher liver weight to body weight ratio than controls ($p<0.01$). The lower dose-group of 50 mg/kg bw/day showed no treatment-related changes. No NOEL can be established due to limited data available.

Sprague-Dawley rat (10 animals per sex and group) received linear alkyl benzene sulphonic acid in feed (0,200, 1000 and 5000 mg/kg feed) for 90 days (corresponding to 8.8, 44 and 220 mg/kg bw). No statistically significant differences were seen in weight gains, food utilisation, or haematological and urinary parameters. Organ to bodyweight ratios as well as macroscopic and microscopic findings were comparable in treated and control groups. No NOEL can be established due to limited data available.

Charles River rats (50 animals per sex and group) received linear alkyl benzene sulphonic acids in feed (0,200, 1000 and 5000 mg/kg feed) for 2 years (dose per kg bw is not given). No statistically significant differences were seen in weight gain and food utilisation during the first 12 weeks. Organ to bodyweight ratios did not show any statistically significant differences when control and highest dose group were compared. At 8 months, male rats in the200 and 1000 mg/kg groups had lower liver weight to bodyweight ratios but this was not seen at later time points and never in the highest dose group. Haematological examinations revealed no treatment related changes. No abnormal macroscopic findings were seen and microscopic findings did not differ between the groups. No NOEL can be established due to limited data available. The highest dose (5000 mg/kg in feed for 2 years) did not show any treatment-related changes.

A published repeated dose toxicity study has been carried out using 6 to 7 months old Beagle dogs (2 animals per sex and group). A linear alkyl benzene sulphonic acid-containing product (15% linear alkyl benzene sulphonic acid) was administered in doses of 0, 10, 100 and 1000 mg/kg bw daily for 6 months by gavage (corresponding to 0, 1.5, 15, and 150 mg linear alkyl benzene sulphonic acid/kg bw). The low dose group showed no treatment-related changes. One female dog in middle dose level group had excessive salivation from the second week forward and one animal regurgitated part of one dose with ensuing sedation and decreased appetite. In the highest dose group, 3 to 4 animals had marked salivation. No animals died. In the highest dose group feed intake was moderately reduced. Marked reduction in weight gain was only seen in the highest dose group (more pronounced in females). No changes were seen in haematological and urinary tests. Eyes and hearing were normal in all groups. In highest dose group mucosal erosions were found in stomach (mainly in cardia) of one male and one female. Haemosiderosis of the spleen was more pronounced in highest dose group. One dog in the same group had small necrotic foci in the pancreas and 2 dogs had some iron-free pigment in kidneys. No NOEL can be established due to small number of animals and limited data available.

According to a WHO report, minimal effects, including biochemical and histopathological changes in the liver, have been reported in subchronic studies in which rats were administered linear alkyl benzene sulphonic acid in the diet or drinking water at concentrations equivalent to doses greater than 120 mg/kg bw per day. These changes appeared to be reversible. In the absence of the original data, no firm conclusion on the data reported in the WHO report can be drawn.

8. Tolerance in dairy cows was studied using commercial teat dip containing 2% linear alkyl benzene sulphonic acid. The product was used post-milking twice daily for 10 days. The product was well-tolerated.

9. Effects on reproduction have been documented in 2 published articles, one of which described a study in rats (10 females and 10 males/group) using only one dose level of linear alkyl benzene sulphonic acids (0 and 100 mg/l drinking water). The data provided are too limited for the assessment.

   Charles River rats (20 males and 20 females/group) received linear alkyl benzene sulphonic acids in feed (0, 0.02, 0.1 and 0.5% daily) in the 3-generation study (dose per kg bw is not given). No gross abnormalities were noted in pups. Rats of the F1 and F2 generations had similar growth patterns and organ to body weight ratios in control and test groups. No abnormalities were seen in histological examinations. In haematological studies, a statistically significant difference (level of significance not indicated) was seen in red blood cell count between control and females of highest test group. F3-weanlings were normal with respect to growth, organ to body weight ratios, macroscopic and microscopic examinations. Haematological values showed no treatment related trend or pattern in this study.

   The studies provided showed no indication of any reproduction toxicity.

10. Teratogenicity data were available from studies conducted using different linear alkyl benzene sulphonic acids in mouse, rat and rabbit using oral, dermal and subcutaneous administration published in five articles. In two mouse studies the exposure periods are not in accordance with the present guidelines. One study in mouse using dermal or subcutaneous administration was carried out using smaller group sizes and exposure periods other than recommended in present guidelines.

    Linear alkyl benzene sulphonic acids (0, 0.2, 2, 300 and 600 mg/kg bw daily) were administered orally to female mice (n = 20), rats (n = 20) (days 6 to 15 of gestation) and rabbits (n = 13) days 6 to 18 of gestation). In all species primary toxic effects in dams were generally associated with disturbance of the gastrointestinal tract (diarrhoea, anorexia, retarded weight gain, weight loss, death). Rabbits were found to be the most susceptible species followed by mice and rats. The two highest dose groups showed maternal toxicity in mice and rabbits resulting in increased foetal loss and reduced litter size. No effects were seen in dams at 2 mg/kg bw in mice and rabbits. In rats, the highest dose caused maternal toxicity also, but did not affect litter parameters. No dose-related trend was seen in foetal weights. No difference was seen in number of major malformations between treated groups and controls. In mice, minor skeletal abnormalities increased to 33.7% in 300 mg/kg bw group compared with 11.7 to 13.3% in controls and lower dose groups. No developmental changes different from controls were seen except an increase in minor skeletal anomalies in the 300 mg/kg bw group in mice. From the highest dose group no viable young were available as a result of marked maternal toxicity.

    When dermal exposure (linear alkyl benzene sulphonic acids in water) was used in mice, rats and rabbits, the two highest doses caused severe skin reactions in mice (50 and 500 mg/kg bw) and rabbits (9 and 90 mg/kg bw). The highest dose in rats (60 mg/kg bw) showed also skin irritation: erythema and oedema with peak response on days 4 to 5. Except for the highest dose group in mice, no treatment related effects were seen in dams and litter data. In mice, a significant ($p<0.05$) increase in embryonic deaths was seen at 50 and 500 mg/kg bw compared with controls. In rats, no significant changes in litter parameters were seen in treated animals. In rabbits, the highest dose group had slightly higher foetal loss and smaller litter size (statistically not significant). No statistically significant differences in anomalies were seen.

    The studies provided showed no indication of any teratogenic potential of the substance.

11. Linear alkyl benzene sulphonic acids (C_{10} to C_{14}, 22.2% solution, 10 to 200 µl/plate) were tested in an *in vitro* test using *Salmonella typhimurium* TA100 and TA98 assays with and without metabolic activation, no mutagenicity was observed. According to the WHO (1996) assessment, mutagenicity has also been tested *in vitro* using *Bacillus subtilis* H17 (rec^+) and M45 (rec^-), *Salmonella typhimurium* TA98 and TA100 (with and without metabolic activation) and *Escherichia coli* WP2*uvr*A, *Salmonella typhimurium* TA1535 and TA1537, and *in vivo* bone marrow aberration test using ICR:ICL mice and chromosomal aberration test using Wistar and Spraque-Dawley rats and ICR mice, micronuclei test in DdY mice and dominant lethal gene induction in ICR:JCL mice. This limited data base has not shown any indication of genotoxic potential *in vitro* and *in vivo* but in the absence of the original data, no firm conclusion on the data reported in the WHO report can be drawn.

12. No formal carcinogenicity study has been carried out. Published long-term toxicity studies using linear alkyl benzene sulphonic acids have not shown increase in malignancies. Linear alkyl benzene sulphonic acids are not considered to have carcinogenic potential.

13. Ten strains of *Escherichia coli, Bifidobacterium spp, Bacteroides fragilis, Eubacterium spp, Clostridium spp, Streptococcus spp, Fusobacterium spp, Lactobacillis spp., Proteus spp.* and *Peptostreptococcus spp.* isolated from human faeces had MIC_{50} and MIC_{90} values greater than 128 µg/ml in the agar-dilution assay. This indicates that bacterial species in human gut are not susceptible to linear alkyl benzene sulphonic acids.

14. The MIC-values for linear alkyl benzene sulphonic acids in milk for *Lactobacillus acidophilus, Lactobacillus rhamnosus* and *Lactobacillus paracasei* was 1.56 µg/ml, for *Lactobacillus bulgaricus* 0.39 µg/ml and for other *Lactobacillus* species tested and for *Streptococcus thermophilus* 0.0078 µg/ml. The study protocol does not define the concentration range studied. Therefore, it is not clear if the lowest MIC values (0.0078 µg/ml) are equal to the lowest concentration tested.

    In the second study effect of linear alkyl benzene sulphonic acids on dairy cultures was tested by measuring the effect on pH in cultures. The NOEL for *Lactococcus lactis subsp. lactis* was 4 µg/ml. The NOELs were higher for other bacterial species tested (*Lactococcus. lactis subsp. cremoris, Lactococcus lactis subsp.lactis biovar diacetylactis, Leuconostoc mesenteroides subsp. cremoris or subsp. dextranicum, Bifidobacterium bifidum, Bifidobacterium longum, Streptococcus thermophilus, Lactobacillus Helveticus, Lactobacillus Delbrueckii subsp. bulgaricus, Lactobacillus Delbrueckii subsp. lactis* and *Lactobacillus acidophilus)*. Dairy cultures were not susceptible to linear alkyl benzene sulphonic acid concentrations present in milk.

15. Based on a published review document, batch tests show that human skin can tolerate contact with solutions containing up to 1% linear alkyl benzene sulphonic acids. No skin sensitisation was induced by linear alkyl benzene sulphonic acids in volunteer studies.

16. Human and environmental exposure has been assessed by WHO in 1996. In the absence of the original data, no firm conclusion on the data reported in the WHO report can be drawn.

17. Based on limited data available, daily exposure to linear alkyl benzene sulphonic acids in general in humans has been estimated to be about 5 mg/person in which the primary route of human exposure is assumed to be through dermal contact and to a lesser extent from the ingestion via drinking water and as a result of residues on utensils and food originating from cleaning agents and personal care products.

18. Due to lacking information and insufficiencies in the studies provided it was not possible to establish NOELs and calculate an ADI for linear alkyl benzene sulphonic acids. However, considering that

    - toxicity has been only seen on high dose levels in all studies available
    - no positive findings have been reported in mutagenicity studies
    - long-term studies have not shown carcinogenic effects,

    the CVMP considered that further toxicity studies were not necessary and that the linear alkyl benzene sulphonic acids residues in foodstuffs of animal origin would not present a risk for human consumers.

19. For the extension to include ovine species in Annex II the following information was taken into account:

20. Absorption was studied in 8 cows treated post-milking with commercial teat dip containing 2% linear alkyl benzene sulphonic acids for 10 days. Plasma samples were taken once a day at days 2, 4, 6, 8 and 10 just before milking during treatment and at days 2, 3, 4 and 5 after the end of treatment. Linear alkyl benzene sulphonic acid concentrations were measured by a validated HPLC/fluorescence method with a limit of detection of 35 µg/l and a limit of quantification of 50 µg/l. The method has shown to be linear in plasma at concentration range of 50 to 500 µg/l. Linear alkyl benzene sulphonic acids were not detected in plasma samples except in two samples (125 µg/l and 53 µg/l). It was concluded that linear alkyl benzene sulphonic acids are not absorbed through the skin in any great extent and do not enter the blood circulation when used for post-dipping.

21. Residues in milk of 8 cows treated post-milking with commercial teat dip containing 2% linear alkyl benzene sulphonic acids by teat dipping for 10 days were determined. Milk samples were taken before milking for analysis at days 2, 4, 6, 8 and 10 during treatment and at days 2, 3, 4 and 5 after the end of treatment. Linear alkyl benzene sulphonic acid concentrations were measured by a validated HPLC/fluorescence method with a limit of detection of 30 µg/l and a limit of quantification of 100 µg/l. The method has shown to be linear in milk at concentration range of 100 to 4000 µg/l. During the treatment, residues were in the range of less than 30 µg/l to 208 µg/l. In most of the samples the concentration was below the limit of detection or the limit of quantification. After the end of treatment residue concentrations in milk were below the limit of quantification except in one milking of one cow at day 2 after the end of treatment showing a concentration of 344 µg/l which could have been caused by external contamination.

Conclusion and recommendation

Having considered that:

- toxicity has been only seen on high dose levels in all studies available ,
- no teratogenic effects have been reported,
- no positive findings have been reported in mutagenicity studies,
- long-term studies have not shown carcinogenic effects,
- for use in footbaths for ovine species the concentration of linear alkyl benzene sulphonates is 100-fold lower than the concentration in bovine teat dips/sprays and will only be used at weekly intervals at the most;

the Committee for Medicinal Products for Veterinary Use recommends the inclusion of linear alkyl benzene sulphonic acids in Annex II of Council Regulation (EEC) No. 2377/90 in accordance with the following table:

Pharmacologically active substance(s)	Animal species	Other provisions
Linear alkyl benzene sulphonic acids with alkyl chain lengths ranging from C_9 to C_{13} , containing less than 2.5 % of chains longer than C_{13}	Ovine	For topical use only