European Medicines Agency
Veterinary Medicines and Inspections

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COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

LASALOCID SODIUM

SUMMARY REPORT

1. Lasalocid is an antibiotic from the group of carboxylic ionophores and is used as sodium salt (CAS No 25999-20-6). Lasalocid is produced by *Streptomyces lasaliensis* and is a mixture of several closely related homologue substances A, B, C, D, E. The sum of the lasalocid homologues B, C, D, E is limited to 10% of the total weight of the active substance, lasalocid. Lasalocid is mainly active against Gram positive microorganisms. Lasalocid sodium is authorised as a feed additive under Council Directive 70/520/EEC for the prevention of coccidiosis in chicken and turkeys. As feed additive the substance is given continuously to chicken from day 0 up to 16 weeks at dose rates of 75 to 125 mg/kg feed with a withdrawal period of 5 days and to turkeys at dose rates of 90 to 125 mg/kg feed up to a maximum age of 12 weeks and with a withdrawal period of 5 days. For veterinary medicine lasalocid sodium is intended to be used in birds for the prevention of coccidiosis caused by *Eimeria spp.* The intended doses in feed are 75 to 125 mg/kg for fattening chickens, 90 to 125 mg/kg for turkeys and 90 to 120 mg/kg for pheasants, partridges and quails.

   Lasalocid sodium is not used in human medicine.

2. Lasalocid sodium is a carboxylic acid ionophore that binds divalent and monovalent cations. The alteration of ionic transport across lipid membranes evokes catecholamine release from cells.

   A positive inotropic effect was observed in dogs following intravenous administration of lasalocid at 1 mg/kg bw and increased coronary and renal blood flow was also demonstrated. *In vitro* data demonstrate reversible effects upon Golgi apparatus in mammalian cellular preparations at 10 µg/ml (incubate) and increased serotonin secretion from platelets at 0.2 µM. No further data on pharmacodynamics were available.

3. Pharmacokinetics after single administration of 1 mg/kg bw ^{14}C-lasalocid orally to mice and rats indicate a rapid absorption of lasalocid. Peak blood concentrations of 0.7 µg/ml and 0.05 µg/ml were obtained at 0.25 hours and 3 hours in mice and rats, respectively. The blood elimination half life was 3 and 4.8 hours for mice and rats, respectively. In both species nearly 90 to 95% of the radioactivity administered was recovered in faeces 48 hours post-administration, indicating an almost complete faecal elimination of lasalocid residues. Lasalocid shows a widespread distribution in rats and mice, affecting many tissues such as muscle, liver, skin, fat, heart, thymus, lung, spleen, etc. The tissue with highest concentrations was the liver with peak concentrations ranging from 2500 to 4000 µg/kg. The urinary excretion of radioactive residues was nearly 1% in both species. After administration of 1 mg/kg bw of ^{14}C-lasalocid, by oral gavage, to biliary duct cannulated rats, 60% of the dose was absorbed from the gastrointestinal tract to the blood. Fifty-eight percent of the administered dose was recovered in bile, indicating that nearly 100% of the absorbed dose was excreted by the biliary duct. Only 1.1% of the administered dose was recovered in urine.

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The pharmacokinetics of ^{14}C-labelled lasalocid was studied in chickens after 16 days administration of 75 of unlabelled lasalocid mg/kg feed followed by a 3-day administration of capsules containing labelled lasalocid at a dose rate of 5 mg/kg bw. Peak plasma concentrations of labelled lasalocid were 5.62 µg/ml and were obtained 2 hours after the last capsule administration. The lasalocid blood elimination half-life was 3 and 48 hours after the last administration the lasalocid concentrations were reduced to 0.39 µg/ml. After analysis of the radioactive contents of edible tissues, the peak concentrations of radioactive residues were 10.3, 0.76, 1.4 and 3 mg/kg in liver, muscle, fat and kidney, respectively. The total radioactive residues recovered in the excreta were 95.6% of the total radioactive dose administered.

After 7-day administration of capsules containing the equivalent of 125 mg/kg bw of ^{14}C-lasalocid in feed to 25 one day old chickens, an average concentration of 0.56 µg/ml of radioactive residues was found in plasma. The plasma radioactive concentration decreased to 0.003 µg/ml after 7-day of the end of the treatment. Eighty-eight percent of the administered labelled lasalocid was eliminated in the excreta at the day of the end of the treatment, the dose fraction excreted 7 days after the end of the treatment was 91%. After the analysis of the contents of lasalocid A in the excreta of the animals after 7 days of the end of the treatment, 74.3 to 76.9% of the administered dose was recovered in the excreta as lasalocid A, 0.8 to 4.1% as lasalocid A homologues, and 0.3 to 4.4% as unidentified components.

4. The acute toxicity of lasalocid has been investigated following oral, dermal, intraperitoneal, and subcutaneous routes of exposure. By oral route the toxicity of lasalocid sodium was assayed in mice, rats, neonatal rats, and rabbits. In these animal species lasalocid showed moderate, acute oral toxicity in mice and adult rats, with oral LD_{50} values of 146 and 122 mg/kg bw, respectively. Lasalocid was highly toxic by oral route in neonatal rats and rabbits, with oral LD_{50} values of 33 and 40 mg/kg bw, respectively. The acute dermal toxicity was assayed in rabbits. Following 24 hours exposure, animals were observed for a further 14 days and lasalocid showed a low acute toxicity with an approximate dermal LD_{50} of 1400 mg/kg bw calculated. The acute intraperitoneal toxicity was investigated in mice and rats. Signs of toxicity included tremors in mice and cyanosis, decreased motor activity, and respiratory depression in rats. Dose-related increases in mortality were observed in both species; LD_{50} values were 68 and 26.5 mg/kg bw for mice and rats, respectively. Acute toxicity via the subcutaneous route was investigated in one mice study and the LD_{50} was calculated to be 140 mg/kg bw. Rabbits and neonatal rats indicated an increased susceptibility to lasalocid.

   The acute oral toxicity of lasalocid to chicken has been investigated in two single-dose studies.

   In the first single-dose study, lasalocid sodium was orally administered to broiler-type chickens, via capsules, at different doses from 39 to 317 mg/kg bw. Toxicity onset was rapid and clinical signs included lethargy, wing droop, resting on hocks, and death generally within 24 hours. Birds dying later showed emaciation and dehydration. Nephromegaly, splenomegaly, and hepatomegaly with scattered foci of necrosis were seen at necropsy. LD_{50} values of 59 and 84 mg/kg bw were calculated for each batch. In the second acute study, birds were gavage dosed with lasalocid sodium, either using 5% acacia gum or 5% of an emulsion product as the vehicle. Oral LD_{50} values of 112 and 84 mg/kg bw, respectively, were calculated for lasalocid sodium in each of the two vehicles. A dose-related decrease in body weight was observed with each vehicle.

5. A higher toxicity of lasalocid in horses and young animals such as calves up to 7 days of age was observed. The estimated oral LD_{50} of lasalocid in horses is 21.5 mg/kg bw. The clinical syndrome includes depression, ataxia, paresis, paralysis, anorexia and recumbency. Cardiac muscle is strongly affected. Doses of 5 to 8 mg/kg bw have caused lethal effects in young calves (single or a few doses).

6. Three 13-week oral toxicity studies were performed in CD rats. These studies used adult rats, weanling rats, and weanlings derived from treated parents. The observed effects on biological parameters were generally consistent across the three rat studies and female animals showed a consistent greater sensitivity to the effects of lasalocid administration than male rats. Common findings included decreased haematocrit and haemoglobin, leucocytosis, small numbers of target cells, a resistance of erythrocytes to lyses by osmotic stress, increased haemosiderin levels in the liver and kidneys, elevated alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanin aminotransferase (ALT), and vacuolation of cardiac muscle.

In the 13-week oral toxicity in weanling rats, lasalocid sodium was administered in the diet at doses of 1, 2, 3, 10 mg/kg bw/day. Neurological examinations, consisting of observations of gait, body position, muscle tone, movement of the legs, and reflexes; ophthalmoscopic examinations, haematology, serum chemistry and urinalysis measurements were performed. At the end of the treatment, all surviving animals were necropsied and examined for gross abnormalities. Reduced bodyweight gain and/or decreased bodyweight, was seen at 10 mg/kg bw, and was more significant in females. Haematological effects, including decreased haematocrit, decreased haemaglobin levels, small numbers or target cell erythrocytes, and slight neutrophilic leucocytosis, were observed in treated animals, but were more extensive in females – haematological effects were seen in females dosed at 2 mg/kg bw and above and in males dosed at 3 mg/kg bw and above. A decrease in the susceptibility of erythrocyte membranes to osmotic stress was seen in some 10 mg/kg bw dosed animals. Biologically significant increases in serum alkaline phosphatase and elevated aspartate aminotransferase (AST) levels were seen in females receiving 10 mg/kg bw. Altered serum electrolyte values were also seen at 10 mg/kg bw. Vacuoles in the cardiac muscle were observed in females treated with 10 mg/kg bw, increased levels of haemosiderin in the kidneys of females and the liver of both sexes, but more apparent in females, at doses of 3 and 10 mg/kg bw. A NOEL of 1 mg/kg bw/day was established, based on the occurrence of a slight decrease in haematocrit and a slight neutrophilic leucocytosis in females at 2 mg/kg bw/day.

A 13-week oral toxicity study was performed in Beagle Dogs (doses: 2, 5, 10 mg/kg bw/day via capsules). General behaviour, signs of toxicity and food consumption were observed daily, and body weights were recorded once per week. Ophthalmoscopic, neurological, electrocardiogram examinations, and haematology and serum chemistry measurements, were performed prior to treatment and at approximately monthly intervals. At the end of the treatment, all dogs were necropsied and examined for gross abnormalities and histology. A slight, but statistically significant decrease in serum chloride values was observed for dogs treated with 5 mg/kg bw and 10 mg/kg bw lasalocid sodium compared to concurrent control. This decrease may be biologically meaningful. Although neurological clinical signs (muscular weakness and/or tremors of the hind limbs, awkward gait) were observed, no histopathological changes were noted in the central nervous system and peripheral nerves of treated dogs. Vacuolation of hepatic parenchymal cells was observed in three females treated with 10 mg/kg bw. Hydropic cellular changes are generally reversible and are non-specific. From the results of this study a NOEL dose of 2 mg lasalocid/kg bw/day can be retained.

A two-years oral chronic toxicity study was performed in Beagle dogs (male and female). Lasalocid was administered with the feed at 10, 35, 180 mg/kg feed. Clinical signs and food consumption were measured and recorded daily. Body weights were measured weekly. Physical, neurological examinations and palpation for tissue masses were performed pre-test and at monthly intervals. The neurological examination included the observation of the following reflexes: righting, patellar, flexor, extensor, visual placing response, corneal and pupillary. Central nervous system observations included: behaviour, movements, reactivity to stimuli and muscle tone. Ophthalmoscopic examinations, electrocardiograms and haematology, clinical chemistry and urinalysis parameters were measured. At the end of the treatment, all surviving dogs were necropsied and examined for gross abnormalities and histology. A slight decrease in food consumption was seen in high-dose animals during the first three months of treatment. Elevated levels of alkaline phosphatase were seen in high-dose animals from month 6 to study termination. Neurological clinical signs were observed (intermittent paralysis of the limbs) in high-dose animals. Nevertheless, no histopathological changes were noted in the central nervous system and sciatic nerves of dosed dogs. Significant reduction in the prostate weight was seen in high-dose males. In ophthalmoscopic examinations, retinal lesions were observed, but the lesions may have had an inflammatory basis. Changes in organ weights were not associated with histopathological lesions. A NOEL of 35 mg lasalocid/kg feed (equivalent to approximately 1 mg/kg bw/day in both sexes) was established, based on the occurrence of transiently reduced food consumption and persistently elevated serum alkaline phosphatase activity, and reduced prostate weight in males at 180 mg lasalocid/kg feed (5 mg/kg bw/day). Dogs appear to be less sensitive to the effects of lasalocid than rats.

7. The oral toxicity of lasalocid to chicken has been investigated in repeated dose studies. In sub-chronic dietary studies, dietary concentrations of up to 125 mg/kg feed (equivalent to approximately 7-11 mg/kg bw/day) produced no observable adverse effects.

   In an study in day-old chickens up to 9 weeks lasalocid sodium was administered via dietary admixture at concentrations from 75 to 375 mg/kg feed. No significant differences in mortality, body weight, feed efficiency, or haematology were seen up to 225 mg/kg feed. In a 13-week study in day-old chicks, increased mortality, reduced body weight gain, and reduced feed efficiency were observed at 225 and 375 mg/kg feed; no effects on haematology were observed. No adverse effects were observed at 75 mg lasalocid/kg feed or 150 mg lasalocid/kg feed (equivalent to approximately 8.8 mg/kg bw/day).

8. A single-generation reproduction study of lasalocid sodium was performed in rats. Males were dosed for 21 days prior to mating and during the 14-day mating period. Females were dosed for 21 days prior to mating, during the 14-day mating period, throughout gestation, and throughout lactation until lactation day 21. F0 body weights and food consumption were measured pre-test and at weekly intervals thereafter. Dams were also weighed at weekly intervals after the day of gestation. After delivery the litters were examined for external anomalies. Pups were counted, weighed and sexed at birth and weighed again at weekly intervals. On lactation day 21, pups were weaned and 60 animals/sex/dose were maintained for a 13-week toxicity study. There was no drug-related effect on clinical signs or mortality, in the F0 or F1 generations. Parental females treated with 10 mg/kg bw weighed significantly less than the control female group from day 0 of gestation onwards. Pup weight at birth was not affected by treatment. At lactation day 4, 7, and 14, weights of pups from the group of parent treated with 10 mg/kg bw were significantly reduced compared with control group pups. By lactation day 21, this difference was reduced and was no longer statistically significant. Abnormalities observed included gastroschisis (1 pup, at 1 mg/kg bw), abnormal flexion of the limbs accompanied by poor muscular coordination (1 pup, at 3 mg/kg bw), bilateral anophthalmia (1 pup, at 1 mg/kg bw) and 1 pup with multiple defects (10 mg/kg bw). There was no effect of treatment on the number of pregnancies or the percent pregnant, the length of gestation, the number of litters born, the average litter size, the average number of implantation sites per litter, the distribution by sex of viable pups, or the gestation, viability or lactation indices. The examination of foetuses was restricted to external observation. A NOEL for all effects of 3 mg/kg bw/day was established, based on the occurrence of reduced maternal weight gain at 10 mg/kg bw/day.

9. A three-generation reproduction and teratology study was performed in rats, comprising the P1 generation and groups of 20 animals per sex of subsequent generations. Animals (male and female) received 0, 10, 35, 120 mg/kg bw of lasalocid sodium with the feed, during nine weeks prior to mating, continuing for three generations. In addition to the assessment of reproductive parameters, females from the first breeding of the first generation (P1F1a), sacrificed on gestation day 13, and the third mating of the third generation (P3F3c), sacrificed on gestation day 19, were used for teratological evaluation. Decreased bodyweight was seen in high-dose females during the growth phases in each generation, but was only statistically significant in P1 females. Slightly decreased body weight was also seen in P1 and P3 high-dose females during gestation, but was not statistically significant. Food consumption of high-dose P1 and P3 females was also slightly decreased during gestation, though this was only significant for P1 dams during the first week. Food consumption during growth phases was unaffected.

   Pregnancy and fertility rates of high-dose groups were consistently decreased, though only significantly in the P3F3b. Weaning and lactation survival indices were also significantly decreased in the P3F3b high-dose offspring. Mean litter sizes at birth were reduced at 120 mg/kg feed in the 1st and 3rd generations. Pup body weights at birth were similar between groups, but at postnatal day four, P2 and P3 pups weighed less than controls. The number of corpora lutea and implantations were decreased in the mid- and high-dose groups in both the P1F1a and P3F3c generations. There was no effect on the incidence of visceral or skeletal abnormalities in the F3c generation, although an increase in delayed ossification was seen at 120 mg/kg feed. No histopathological findings were seen in the F3b generation. The NOEL for this study was 10 mg lasalocid/kg feed (approximately 0.5 to 0.8 mg lasalocid/kg bw/day).

10. A preliminary embryo-foetal toxicity study was performed in female New Zealand White rabbits. The animals received 0, 1, 2, 4 mg/kg bw/day. Treatment was administered daily from gestation days 6 through 28. Animals were examined daily for clinical signs and were checked twice daily for viability. Foetuses were examined for external abnormalities and the total weight of live foetuses per litter recorded. A dose-dependent decrease in bodyweight gain was seen. A decrease in the number of mean live implants, caused by an increase in the number of early and late embryonic deaths, was seen at 4 mg/kg bw. Foetal weight was decreased at all doses. One animal treated with 4 mg/kg bw had a shortened tail with a small skin flap.

    A second study with female New Zealand White rabbits was conducted. The animals were treated with 0, 0.5, 1, 2 mg/kg bw/day by oral route (gavage) from days 6 through 28 of gestation. Animals were examined for clinical signs daily. Body weights and food consumption were recorded daily from day 4 or 5 of gestation. Animals were sacrificed and necropsied and foetuses examined on day 29 of gestation. No treatment-related maternal mortality was seen. Treatment of rabbits with lasalocid sodium was associated with a dose-related decrease in food consumption at 1 and 2 mg/kg bw. This led to a reduction in faecal output and significantly reduced body weight/weight gain at 2 mg/kg bw. At 2 mg/kg there was a slight increase in the incidence of foetuses with corneal opacity. At 1 and 2 mg/kg bw/day there was a slight increase in the incidence of pale spleen. Only at 2 mg/kg bw/day the incidence of foetuses with jungals connected to maxilla was greater than control. The incidence of foetuses at 2 mg/kg bw/day with complete 13^{th} supernumerary ribs and displaced pelvic girdle was marginally greater than control. At 2 mg/kg bw/day there was an increase in the incidence of foetuses with incomplete ossification. The NOEL for foetal toxicity was 0.5 mg lasalocid/kg/day (based on pregnancy performance and foetal body weight). The NOEL for maternotoxicity is considered to be 0.5 mg lasalocid/kg/day, based on systemic toxicity.

11. Lasalocid was tested in a series of *in vitro* mutagenicity test systems. In the rec-assay, Wilkins-Chalgren lasalocid was investigated for its potential for killing repair-deficient cells. Concentrations of 1, 10, and 100 µg/disc of lasalocid sodium, dissolved in dimethyl sulfoxide, were incubated with *Bacillus subtilis* M45 (DNA-recombination-deficient) and H17 (DNA recombination-proficient. After 24 hours incubation, the difference between the growth inhibitory zones for the two strains, for each concentration of lasalocid, was less than both the positive and negative controls. Hence, lasalocid sodium showed no DNA damaging effect in this assay. In the bacterial reverse mutation test assay, the mutagenic potential of lasalocid sodium was investigated, both in the presence and absence of metabolic activation, in *Salmonella typhimurium* strains TA1535, TA 1537, TA 98, and TA 100, and *Escherichia coli* strains trp^-B/r WP2 and trp^-WP2 hcr^-. The results of the bacterial reverse mutation assay indicate that lasalocid sodium, at concentrations up to 2000 µg/kg/plate, does not induce gene mutations in the strains tested, either in the presence or absence of metabolic activation.

    In the yeast assay, *Saccharomyces cerevisiae* D7 was used to detect the frequencies of gene conversion at the trp5 locus, mitotic recombination and other mitotic segregations using the ade 2 marker, and reverse mutations at the ilv 1-92 mutant allele. Lasalocid concentrations of 0, 0.05, 0.17, 0.50, 1.67, and 5 mg/ml were used, both in the presence and absence of metabolic activation. Lasalocid did not induce gene conversion, reverse mutation or mitotic crossing-over, either with or without metabolic activation.

    Lasalocid was assessed for mutagenic activity at the hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus in Chinese hamster lung V79 cells. Cells were incubated with lasalocid at concentrations of 1 to 20 µg/ml in the absence of metabolic activation and 1 to 60 µg/ml in the presence of metabolic activation. Cytotoxicity was observed with high concentrations of lasalocid preventing assessment. The results of this study indicate that lasalocid does not induce gene mutation in the cultured mammalian cells used, either with or without metabolic activation.

    Lasalocid was assessed for genotoxicity in the unscheduled DNA synthesis assay in primary cultures of male rat hepatocytes. Lasalocid concentrations of 0 to 12.5 µg/ml were tested initially, followed by concentrations of 0 to 5 µg/ml in a second assay. Lasalocid sodium was strongly cytotoxic with no viable cells available for assessment at concentrations of more than or equal to 4 µg/ml. Altered morphology occurred at 1 µg/ml. Lasalocid did not induce DNA damage

resulting in repair, either in the presence or absence of activation, at concentrations up to those producing cytotoxicity. Therefore, no genotoxicity can be concluded from this assay.

Lasalocid was assessed for its potential to induce chromosomal aberrations in human peripheral lymphocytes. It was tested three times in the absence of metabolic activation at concentrations of 0 to 8 µg/ml; concentrations of 0 to 10 µg/ml were also tested in the presence of metabolic activation. In each of the tests, cytotoxicity prevented assessment at the higher lasalocid concentrations. However, for lasalocid, at concentrations of 2 to 8 µg/ml, no clastogenicity can be concluded from this assay, either with or without metabolic activation.

It was concluded that lasalocid was not mutagenic.

12. Two *in vivo* rodent carcinogenicity studies in mouse and in rat have been reported. In a mouse study, lasalocid sodium was administered at concentrations of 0, 10, 35, and 120 mg/kg feed during two years, except during the first five weeks when low- and mid-dose concentrations of 20 and 60 mg/kg feed were used. No clinical signs or ophthalmic effects were observed, there was no effect on mortality, and no consistent effects on body weight or food consumption were seen. In males, and in females surviving until sacrifice, no treatment-related pathology or histopathology were observed and the incidence of neoplasms was similar across all groups. In females that died or were euthanised during the study, an increase in the incidence of lymphosarcoma was seen in the low- and high-dose groups (nine and ten cases, respectively, compared to three and five cases in the two control groups). However, no increase was seen either in decedents of the mid-dose group (four cases), or in the approximately 50% of animals from each treatment group that survived to the end of the study. No increased incidence of lymphosarcoma was detected in the treated males or terminal sacrificed females.

    In the rat study, lasalocid sodium was administered with the feed, at concentrations of 0, 10, 35, and 120 mg/kg feed. After one week, animals were mated. Treatment was continued through mating, gestation, and lactation. At weaning, F1 animals were selected for continued treatment for a further 130 weeks. Whilst survival in the study was low (21.8 to 43.6%) at study termination, survival rates at week 104 were above 50% and the study is therefore considered adequate. Decreased serum blood urea nitrogen levels were observed in all treatment groups, including the low-dose group at weeks 26 and 78. No histopathological changes were noted in the kidneys. During the week 27 through 53 period, an increase in the number of mid- and/or high-dose females (higher incidence in high-dose) and high-dose males with slow grasping or righting reflexes was noted. There was no treatment-related pathology or histopathology and the incidence of neoplasms was similar across all groups. A conservative NOEL for all effects of 10 mg lasalocid/kg feed (equivalent to approximately 0.5 mg/kg/day in males and 0.6 mg/kg/day in females) was established, based on increased blood glucose and reduced urea-nitrogen concentrations, and increased adrenal weight at 35 mg/kg feed.

13. Lasalocid was evaluated for skin and ocular irritation in New Zealand rabbits. The studies suggest that lasalocid is non irritating to the skin, but is an ocular irritant. The sensitisation potential of lasalocid has been investigated in a guinea pig maximisation test. Lasalocid -induced animals showed no greater response to challenge with lasalocid than animals induced with test vehicle only.

14. No specific studies on neurotoxicity have been provided. Published data showed that lasalocid induced histopathological changes in peripheral nerves in birds. Biophysical or biochemical changes in the nervous system were also described in dogs and rats. Acute and subchronic and chronic studies in dogs have demonstrated neuromuscular effects, evoked beginning with doses of 5 mg lasalocid/kg bw or above. At these doses levels, effects appeared to be transient and without histopathological findings. In one chronic study in rats an effect on grasping and righting was reported at several time points, at dietary doses corresponding to about 2 and 6 mg/kg bw.

    Overall, the neurotoxicity effects were not considered to present a significant risk to the safety of consumers.

15. No specific data on humans have been provided. Lasalocid is not used in human medicine.

16. The toxicological ADI for lasalocid was calculated based on the NOEL (0.5 mg/kg/day) from the 2-year chronic oral toxicity study in rat and the maternal toxicity study in rabbits and applying an uncertainty factor of 200 due to the limited data in respect to neurotoxicity.

17. Three sensitive bacterial strains (*Staphylococcus aureus*, *Enterococcus faecalis*, and *Clostridium perfringens*) were serially cultured 20 times in the presence and absence of sub-lethal concentrations of lasalocid. The potential of cross resistance was also investigated. A non significant selective resistant pressure in the presence of lasalocid was observed. No cross resistant selection, on a panel of eight antimicrobials, was observed.

    The Minimum Inhibitory Concentrations (MIC) of lasalocid sodium were determined against 15 strains of anaerobic bacteria representing the human intestinal microbiota. These comprised five isolates each of *Bacteroides spp*., *Fusobacterium spp*. and *Peptostreptococcus spp*. The test system was based on standardized agar dilution MIC methodology, as described in NCCLS guidelines. MIC determinations were performed using agar at pH 7.1, pH 6.0 and pH 8.5, conditions representing the pH range of the human intestinal ecosystem. For 14 of the 15 bacterial strains tested, the greatest difference between MIC results obtained at any two pH levels equated to two doubling dilutions in the MIC series. For a single *Fusobacterium* strain, lasalocid MIC obtained at pH 8.5 was three dilutions higher than that obtained at pH 7.1. However, the MIC obtained at pH 6.0 was also higher than that obtained at pH 7.1, by a factor of two dilutions. Thus, there was no clear trend with regard to variations in lasalocid MIC against this strain with agar pH.

    A faecal binding study was conducted with lasalocid. The effect of faecal binding on the antimicrobial activity of lasalocid was studied by incubating selected lasalocid concentrations (0 to 100 µg/ml) with sterile pooled human faeces at concentrations of 0, 10, 20 and 50% w/v. After incubation of each combination for up to eight hours, faecal solids were removed by centrifugations and the supernatant liquid inoculated with a lasalocid-susceptible *Enterococcus faecalis* strain and incubated for 48 h. Antibacterial activity in each inoculated preparation was determined after 24 hours and 48 h by presence or absence of bacterial growth. Without prior incubation with faeces, a lasalocid concentration of 1 µg/ml consistently inhibited *E. faecalis* growth at each sampling point. Following brief incubation with 10% faeces, the initial lasalocid concentration required to inhibit *E. faecalis* growth increased to more than 100 µg/ml. This increase indicates that more than 99% of the lasalocid concentration was bound to faeces, reducing the concentration available in the supernatant. In the presence of all faecal concentrations, the degree of lasalocid binding remained consistent throughout the 8-hour incubation period and is regarded as irreversible. As there are presently no accepted and validated protocols for this type of investigation and due to concerns about the limit of detection of the analytical method, the number of strains tested, the presence of lasalocid on the supernatant, the lack of information about the binding of lasalocid and faeces in suspension and whether this binding is irreversible as well as the lack of positive controls, the retained fraction of the oral dose available for microorganisms was established as 0.1.

    The Minimum Inhibitory Concentrations (MIC) of sodium lasalocid against 84 bacterial strains representative of human gut microbiota were determined. Ten strains of *Bifidobacterium* spp., 10 strains of *Eubacterium* spp., 10 strains of *Clostridium* spp., 10 strains of *Peptostreptococcus* spp., 3 strains of *Lactobacillus acidophilus*, 9 strains of *Enterococcus* spp., 10 strains of *Streptococcus* spp., 3 strains of *Bacteroides fragilis*, 7 strains of *Fusobacterium* spp., 3 strains of *Escherichia coli*, 3 strains of *Proteus* spp., and 6 strains of *Salmonella enterica* serovar Enteritidis or serovar Typhimurium were tested. The methodology employed was MIC agar dilution as described by the NCCLS for anaerobic bacteria (M11-43). There were differences between MIC_{50} for the different media that could be explained by binding of lasalocid to proteins, MIC_{50} on Wilkins-Chalgren media would be considered the most appropriate for the assessment of the Minimal Inhibitory Concentration of sodium lasalocid. In an additional study the MIC of sodium lasalocid against 30 bacterial strains, representing the normal human intestinal microbiota, was determined. Ten isolates each of *Bacteroides spp*., *Fusobacterium spp*. and *Peptostreptococcus spp*. were sourced from the faecal microbiota of healthy unmedicated humans. The test system was standardized agar dilution MIC methodology as described for NCCLS.

With the aim of the determination of an overall microbiological ADI, the MIC data for *Fusobacterium spp*., *Escherichia coli*, *Proteus* spp., and *Salmonella enterica* were excluded due to lack of sensitivity and the values for *Bifidobacterium* spp., *Eubacterium* spp., *Clostridium* spp., *Peptostreptococcus* spp., *Lactobacillus acidophilus, Enterococcus* spp., *Streptococcus* spp., and *Bacteroides fragilis* from both studies were used to determine the overall MIC_{50}. The lower 10% confidence limit ($CL10\%_{lower}$) of MIC_{50} was 0.134 µg/ml.

For the establishment of the microbiological ADI, the following standard formula agreed by the CVMP was used:

$$\text{ADI (µg/kg bw)} = \frac{\dfrac{\text{geometric mean } MIC_{50} \times CF2}{CF1} \text{ (µg/ml)} \times \text{daily faecal bolus (220 g)}}{\text{fraction of an oral dose available for microorganisms} \times \text{weight of human (60 kg)}}$$

And therefore the microbiological ADI was calculated as indicated below:

$$\text{ADI} = \frac{\dfrac{0.134 \times 1}{1} \times 220}{0.1 \times 60} = 4.91 \text{ µg/kg bw i.e. } = 294.6 \text{ µg/ 60 kg person}$$

The following assumptions were made:

- CF1 = 1 because no resistant selection has been demonstrated under *in vitro* or *in vivo* conditions for lasalocid or for other ionophore antibiotics,
- CF2 = 1 because, no justification for a higher value could be accepted,
- the retained fraction of the dose available was 10%
- 220 g was the weight of the daily faecal bolus.

A microbiological ADI of 4.91 µg/kg/day bw (i.e. 294 µg/person) was calculated.

18. Considering that the toxicological ADI is lower than the microbiological ADI, the toxicological ADI of 2.5 µg/kg/day was considered the overall ADI for the assessment of the safety of the consumer (150 µg/person/day).

19. The plasma kinetics in chickens and rats were similar. However, limited data on chicken's metabolism profile were provided making it difficult to compare the metabolic fate of lasalocid between chickens and laboratory animals.

20. The residues of ^{14}C labelled lasalocid were studied in chickens after 16 days of administration of 75 mg/kg of unlabelled lasalocid in feed followed by a 3 days administration of capsules containing labelled lasalocid at a dose rate of 5 mg/day. After analysis of the radioactive contents of edible tissues, the peak concentrations of radioactive residues were 10300, 760, 1400 and 3000 µg/kg in liver, muscle, fat and kidney respectively, and they were obtained 2 hours post-treatment suppression.

    After 7-day administration of 127 mg/kg feed ^{14}C labelled lasalocid to broiler chickens, the total radioactive residue concentration was compared to the lasalocid A concentration. Eight hours after treatment, the radioactive concentration found in liver was 2.01 µg/ml while the concentration of lasalocid A was 0.094 µg/ml. The lasalocid A in liver represents 4.6% of the radioactive residues.

Thirty Cornish Cross chickens were treated with 125 mg/kg feed of unlabelled lasalocid for 34 days in feed followed by 21 days of treatment with 132 mg/kg feed of radiolabelled lasalocid. Groups of animals were sacrificed at 0, 1, 2, 3, 4 and 5 days after treatment suppression and edible tissues were collected. The radioactive tissue residues were quantified. At 0 days after treatment the fat, skin, kidney, muscle and liver radioactive concentrations were, respectively, 860, 1590, 2480, 610 and 11 930 µg/kg. Twenty four hours after the treatment the fat, skin, kidney, muscle and liver radioactive concentrations were, respectively, 140, 220, 360, 60, and 2630 µg/kg. Forty-eight hours after treatment the fat, skin, kidney, muscle and liver concentrations were, respectively, 60, 130, 230, 30, and 1720 µg/kg. The radioactive residue concentration in skin, fat, muscle and kidney were below 200 µg/kg at 3 to 5 day after treatment. The radioactive concentrations in liver at 3, 4 and 5 days post-treatment were, 1590, 1370 and 1150 µg/kg respectively, indicating that this tissue shows a relatively slow elimination pattern of the residues.

Unlabelled lasalocid at a concentration of 90 mg/kg feed was administrated to 40 broiler chickens during 14 days. Blood, liver and muscle lasalocid concentrations were quantified, using an ELISA technique, 0 to 7 days after treatment. The elimination half life of lasalocid was 11, 36, and 41 hours for serum, liver and muscle, respectively. Seven days after treatment, only liver showed lasalocid concentrations above 10 µg/kg.

After 7-day administration of capsules containing the equivalent of 125 mg/kg of ^{14}C-lasalocid in feed to 25-day broiler chickens, the total radioactive concentrations in edible tissues were quantified. Also, the lasalocid A concentrations were quantified using an HPLC technique. After analysing the total tissue residues, some lasalocid A homologues were found and up to seven unknown metabolites were detected. At 0 h withdrawal time, the total radioactive residues were 1.22, 0.40, 0.08 and 0.43 µg/g in liver, kidney, muscle and skin+fat, respectively. The lasalocid A concentrations at the same time were 0.24, 0.122, 0.04 and 0.21 µg/g for liver, kidney, muscle and skin+fat, respectively. The ratios for lasalocid A concentrations to total radioactive concentration, at 0 h withdrawal time, were 0.22, 0.41, 0.55, and 0.52 for liver, kidney, muscle and skin+fat, respectively. At 24 post dose, the marker to total residue ratio were 0.086, 0.142 and 0.283 for liver, kidney and skin+fat, respectively. Lasalocid A was confirmed as the marker residue. At 0 h and 24 h withdrawal period the total radioactive residue consumption, taking into account the food basket proportions, represents 126% and 68% of the ADI, respectively.

21. Based on the total tissue residue concentrations found at 0 hours withdrawal period, MRLs of 100 µg/kg for liver and skin+fat, and of 50 µg/kg for kidney and 20 µg/kg for muscle can be retained. The tissue residues of lasalocid in pheasants and quail were studied. After administration of 90 mg/kg of lasalocid for 27 days to quails, the tissue concentrations (skin, muscle and skin + fat) were analysed. The highest tissue concentrations were found in skin (298.3 µg/kg, 55 µg/kg, 30.8 µg/kg and 33.7 µg/kg at 0, 3, 6 and 9 days after treatment, respectively) and they were ten fold higher than the muscle lasalocid concentrations. After 7 day administration of medicated feed with 132 mg/kg of lasalocid sodium to pheasants, the liver and skin+fat lasalocid A concentrations were 28.5 and 30.7, respectively. Lasalocid A, as marker residue, was found in quail and pheasant tissues. The studies provided were according to the requirements for extrapolation to poultry of the Notes for Guidance on Risk Analysis Approach for Residues of Veterinary Medicinal Products in Food of Animal Origin (EMEA/CVMP/187/00-FINAL) and on the Establishment of Maximum Residue Limits for Minor Animal Species (EMEA/CVMP/153a/97-FINAL). This method should be applicable to other poultry and therefore from this aspect extrapolation to all poultry is possible.

22. The analytical method used to quantify the lasalocid residues (lasalocid A) involve a liquid-liquid extraction followed by an HPLC analysis with a fluorimetric detection. The HPLC method describes the detection of lasalocid A in chicken edible tissues (muscle, liver, kidney, and skin + fat). The method is applicable in the range of 20 to 500 µg/kg for all tissues, with a limit of quantification of about 20 µg/kg for all tissues and limits of detection of 0.4 µg/kg for muscle, 1.6 µg/kg for liver, 2 µg/kg for kidney, and 0.5 µg/kg for skin + fat. The specificity of the analytical method against other ionophores (e.g. monensin, salinomycin and narasin) and the accuracy and precision were calculated according to the proposed MRLs. The analytical method is applicable for the lasalocid A tissue residue quantification in quail and pheasants.

23. The EFSA Scientific Panel on Additives and Products or Substances used in animal feed also assessed a product that contains lasalocid sodium further to a request from the European Commission to re-evaluate the substance and give advice on its efficacy and safety. The EFSA Scientific Panel concluded that the data provided proved insufficient to give conclusive answers to several of the questions raised. A lowest NOEL of 0.5 mg/kg bw/day was established from a 2-year chronic toxicity study in rats and a maternal toxicity study in rabbits to which applying a safety factor of 100 would lead to an ADI of 5 µg/kg bw/day. However, it was considered that the similarity between the metabolic profiles of lasalocid sodium in the laboratory animals (rat) and chicken had not been thoroughly established and therefore concerns remained of the adequacy of the evaluation of residues in chicken tissues. The liver was established as the target tissue, a marker residue could not be established nor MRLs. The Commission considered that the re-evaluation of lasalocid sodium showed that the conditions laid down in Directive 70/524/EEC were satisfactory for a specific product containing lasalocid sodium at 15% and that therefore should be authorised for 10 years. Commission Regulation (EC) No 1455/2004 published such considerations.

Conclusion and recommendations

Having considered that:

- an ADI of 2.5 µg/kg/day (i.e. 150 µg/ 60 kg bw person/day) has been established based on the toxicological studies,
- lasalocid A was retained as the marker residue. The marker residue represents 22%, 41%, 55%, and 52% in liver, kidney, muscle and skin+fat, respectively of the total residues at 0 hours after treatment,
- the marker residue lasalocid A can be found in quail and pheasant tissues,
- a validated routine analytical method for determining the concentrations of lasalocid A in chickens is available,
- an analytical method for the monitoring of residues in other poultry species was available,
- in view of the low level of residues in muscle at 0 hours the MRL for muscle was established at the limit of quantification of the analytical method,
- no residue studies or analytical method were provided for eggs, so the substance will not be allowed to be used in birds from which eggs are produced for human consumption;

the Committee for Veterinary Medicinal Products recommends the inclusion of lasalocid in Annex I of Council Regulation (EEC) nº 2377/90 in accordance with the following table:

Pharmacologically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions
Lasalocid	Lasalocid A	Poultry	20 µg/kg 100 µg/kg 100 µg/kg 50 µg/kg	Muscle Skin + fat Liver Kidney	Not for use in animals from which eggs are produced for human consumption

Based on these MRL values, the daily intake will represent about 49.4% the ADI.