WHO FOOD ADDITIVES SERIES: 69

Prepared by the Seventy-eighth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA)

Toxicological evaluation of certain veterinary drug residues in food

WHO Food Additives Series No. 72,

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World Health Organization, Geneva, 2014

Food and Agriculture Organization of the United Nations

World Health Organization

LASALOCID SODIUM

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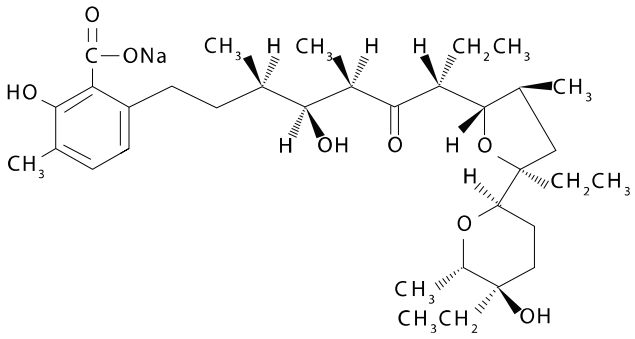
Fig. 1. Structure of lasalocid sodium (lasalocid A)



1. EXPLANATION

Lasalocid sodium (Chemical Abstracts Service No. 25999-20-6) is produced by *Streptomyces lasaliensis* and is a mixture of several closely related homologues: A, B, C, D and E. Lasalocid homologues B, C, D and E make up no more than a total of 10% of the total weight of the active substance.

Lasalocid sodium, a divalent polyether ionophore antibiotic, is approved for continuous use to control coccidiosis in poultry species at concentrations of 7.5–125 mg/kg feed. It is approved to protect against *Eimeria* species in broilers and replacement pullets, turkeys, pheasants and quails.

The mechanism of action of lasalocid and other ionophores has been extensively investigated and reported. Like other carboxylic polyether ionophores, lasalocid disturbs ionic homeostasis, leading to osmotic lysis of coccidia.

Lasalocid sodium has not previously been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated lasalocid sodium at the present meeting at the request of the Twentieth Session of the Codex Committee on Residues of Veterinary Drugs in Foods (FAO/WHO, 2012) with a view to establishing an acceptable daily intake (ADI) and recommending maximum residue limits (MRLs) in poultry tissues and eggs.

The structure of lasalocid sodium (International Union of Pure and Applied Chemistry name: sodium 6-[(3*R*,4*S*,5*S*,7*R*)-7-[(2*S*,3*S*,5*S*)-5-ethyl-5[(2*R*,3*R*,6*S*)-5-ethyl-5-hydroxy-6-methyltetrahydro-2*H*-pyran-2-yl)]tetrahydro-3-methyl-2-furyl)]-4-hydroxy-3,5-dimethyl-6-oxononyl)]-2,3-cresotate) is shown in Fig. 1.

The present Committee considered data on pharmacodynamics, pharmacokinetics, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, carcinogenicity and microbiological safety. The original studies provided by the sponsor were all performed in the 1970s and 1980s; consequently, a number of them do not conform to the standards in force today. Several were performed before the introduction of good laboratory practice (GLP) and provide relatively limited reporting. Nevertheless, the overall package of data is considered sufficient to allow the derivation of a robust ADI. In addition

to a submission from the sponsor, a literature search was conducted using the Embase database and the following keywords: lasalocid, Ro 2-2985, X-537A, toxicity, cytotoxicity and neurotoxicity. Seventy-seven references were obtained and manually screened. Five of these reports are cited in the current monograph and were not included in the dossier provided by the sponsor.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Mice

In a study that pre-dated the implementation of GLP, 35 adult male Charles River CD-1 mice were administered a single dose of [^{14}C]lasalocid sodium at 1 mg/kg body weight (bw) by oral gavage. Groups of five animals were killed at 15 and 30 minutes and 1, 3, 6, 24 and 48 hours after dosing. Radioactivity was determined using liquid scintillation counting in whole blood, brain, carcass (i.e. all tissues not collected as individual tissues), faeces, fat, heart, kidney, large intestine contents, large intestine tissue, liver, lung, small intestine contents, small intestine tissue, spleen, stomach contents, stomach tissue, thymus and urine. Urine and faeces were collected as separate samples for each mouse over 24-hour periods.

Whole blood radioactivity levels were highest at 15 minutes after administration (at which point the levels accounted for 1.41% of the administered radioactivity). Radioactivity levels in whole blood were below the minimum detectable level at 24 hours and beyond. The half-life of elimination of radioactivity in whole blood was 3 hours.

Radioactivity was observed in all tissues examined, and levels were highest at 1 hour after administration. Radioactivity levels were highest in liver (at 1 hour after administration, residues in liver accounted for approximately 17.5% of the administered radioactivity). In all other tissues, radioactivity levels at 1 hour accounted for less than 0.5% of the administered dose and decreased in the order heart > lung > brain = kidney > spleen = thymus. Levels in fat were highest at 3 hours after administration (accounting for approximately 0.1% of the administered radioactivity). With the exception of liver, radioactivity levels were below the minimum detectable level in all of the above tissues by 24 hours after administration. Residues in liver remained detectable at 48 hours after administration.

Residues in the remaining carcass were detectable between 15 minutes and 6 hours after administration and peaked at 3 hours.

In the gastrointestinal tract tissues, radioactivity levels were highest in stomach contents 15 minutes after administration; highest in stomach tissue at 30 minutes after administration; highest in the small intestine contents between 15 minutes and 3 hours after administration; highest in small intestine tissue at 15 minutes after administration; highest in large intestine contents at 6 hours after administration; and fairly steady in large intestine tissue over the first 6 hours.

High levels of radioactivity were seen in the faeces from 6 to 24 hours after administration, with approximately 95% of radioactivity being excreted in faeces by 24 hours. Radioactivity was observed in urine between 3 and 24 hours after administration and accounted for less than 1% of the administered dose (Laurencot et al., 1979a).

In a study that pre-dated GLP implementation, 40 adult male Charles River CD-1 mice were administered seven daily doses of [^{14}C]lasalocid sodium at 1 mg/kg bw by oral gavage. Groups of five animals were killed at 15 and 30 minutes and 1, 3, 6, 24 and 48 hours after the last dose. Radioactivity was determined using liquid scintillation counting in whole blood, brain, carcass (i.e. all tissues not collected as individual tissues), faeces, fat, heart, kidney, large intestine contents, large intestine tissue, liver, lung, small intestine contents, small intestine tissue, spleen, stomach contents, stomach tissue, thymus and urine. Urine and faeces were collected as separate samples for each mouse over 24-hour periods. The remaining five animals were also killed at 48 hours after the last dose, but only their blood, urine and faeces were assayed.

Whole blood radioactivity levels were highest at 30 minutes after the last dose (at which point levels accounted for approximately 0.25% of the administered dose). Radioactivity levels in whole blood at 24 hours and beyond were below the minimum detectable level. The half-life of elimination of radioactivity in whole blood was 3 hours.

Radioactivity was observed in all tissues examined and was highest during the 3-hour period following the last administration. At 3 hours after administration, average radioactivity levels were highest in liver (accounting for almost 2% of the administered dose). In all other tissues, radioactivity levels at 3 hours accounted for less than 0.5% of the administered dose and decreased in the order heart > lung > brain > thymus > kidney > spleen > fat. With the exception of liver, levels of radioactivity were below the minimum detectable levels in all of the above tissues by 24 hours after administration. Residues in liver remained detectable at 48 hours after administration.

Residues in the remaining carcass were detectable between 15 minutes and 6 hours after administration and peaked at 3 hours.

In the gastrointestinal tract tissues, radioactivity was highest in stomach contents 15 minutes after the last administration; highest in stomach tissue at 15 minutes after the last administration; highest in the small intestine contents at 1–3 hours after the last administration; highest in small intestine tissue at 15 minutes after administration; highest in large intestine contents at 6 hours after the last dose; and highest in large intestine tissue at 6 hours after the last dose.

Radioactivity levels in faeces were roughly similar during each 24-hour period during the dosing period and for the 24 hours after the last administration. Approximately 95% of radioactivity was excreted in faeces by 24 hours after the last dose. Radioactivity levels in urine were also roughly similar during each 24-hour collection period during the dosing interval and during the 24 hours after the final dose. Excretion via the urine accounted for less than 1% of the total administered dose (Laurencot et al., 1980).

In a study reported to have been performed in compliance with GLP (a signed quality assurance statement was provided), 26 Charles River CD-1 mice (13 males and 13 females) were administered [^{14}C]lasalocid sodium as single oral (gavage) daily doses of approximately 1 mg/kg bw for 7 days. The drug was administered in 30% aqueous ethanol. Ten additional mice (five males and five females) served as untreated controls. Urine and cage wash were collected at 24-hour intervals for each cage during treatment and for up to 4 hours following the last dose. Faeces were collected from each cage at 24-hour intervals over the 7-day period. At 4 hours after the last dose, mice were killed. Radioactivity was determined in urine, cage wash, faeces and liver by liquid scintillation counting.

After the first oral dose, 96.68% of the administered radioactivity was excreted within 24 hours, with 89.75% detected in faeces, 1.44% in urine and 5.49% in cage wash. By 4 hours after the last dose, 77.08% of the dose was excreted in faeces, 1.01% in urine and a further 2.61% in cage wash (Hawkins, Elsom & de-Salis, 1987a).

Samples of pooled lyophilized livers and faeces were kept for metabolic profiling, which was undertaken as part of a separate study (Laurencot & Weiss, 1987) reported in section 2.1.2 below.

In a study that pre-dated GLP implementation, 10 adult Charles River CD-1 mice (5 males and 5 females) were administered [^{14}C]lasalocid sodium as a single oral (gavage) dose of 1 mg/kg bw in 10% ethanol. Urine and faeces were collected (separately) from individual animals over the following intervals: 0–4 hours, 4–8 hours, 8–12 hours, 12–24 hours, 24–48 hours and 48–72 hours. Radioactivity was determined using liquid scintillation counting.

In both male and female mice, 96% of the administered radioactivity had been excreted in faeces by 72 hours after dosing, with the bulk of it excreted within the first 24 hours (approximately 95% in males and 93% in females). Peak radioactivity levels were seen in faeces of males collected between 8 and 12 hours after administration and in faeces of females collected between 4 and 8 hours after administration. In both males and females, radioactivity in urine (over the entire 72-hour period) accounted for less than 1% of the administered radioactivity (Westheimer & Hutchinson, 1978a).

(b) Rats

In a study that pre-dated GLP implementation, 35 adult male Charles River CD rats were administered a single dose of [^{14}C]lasalocid sodium at 1 mg/kg bw by oral gavage. Groups of five animals were killed at 15 and 30 minutes and 1, 3, 6, 24 and 48 hours after dosing. Radioactivity was determined using liquid scintillation counting in whole blood, brain, carcass (i.e. all tissues not collected as individual tissues), faeces, fat, heart, kidney, large intestine contents, large intestine tissue, liver, lung, small intestine contents, small intestine tissue, spleen, stomach contents, stomach tissue, thymus and urine. Urine and faeces were collected as separate samples for each rat over 24-hour periods.

Whole blood radioactivity levels were highest at 3 hours after administration (accounting for 0.122% of the administered radioactivity). Radioactivity levels in whole blood were below the minimum detectable level at 48 hours after administration. The half-life of elimination of radioactivity in whole blood was 4.8 hours.

Radioactivity was observed in all tissues examined. Average radioactivity levels were highest in liver (approximately 10% of the administered dose at 6 hours). In all other tissues, radioactivity levels accounted for less than 0.1% of the administered dose at all time points (levels decreased in the order lung > fat > kidney > spleen > heart > thymus > brain). Levels of radioactivity peaked in the above tissues between 15 minutes and 6 hours after administration. Low levels of radioactivity were still present in a number of tissues at 48 hours after administration.

Residues in the remaining carcass were detectable between 15 minutes and 6 hours after administration and peaked at 15 minutes.

In the gastrointestinal tract tissues, radioactivity levels were highest in stomach contents 15 minutes after administration; highest in stomach tissue at 30 minutes; highest in the small intestine contents at 3 hours after administration; highest in small intestine tissue also at 3 hours; highest in large intestine contents at 6 hours after administration; and highest in large intestine tissue also at 6 hours.

High levels of radioactivity were seen in the faeces at 24 hours after administration, with approximately 85% of radioactivity being excreted in faeces by 24 hours. A further 9% was excreted in faeces between 24 and 48 hours. Radioactivity was observed in urine between 15 minutes and 24 hours after administration and accounted for less than 1% of the administered dose (Laurencot et al., 1979b).

In a study that pre-dated GLP, 52 adult Charles River CD rats (26 males and 26 females) were administered unlabelled lasalocid sodium in the diet for 2 weeks. During the 1st week, the concentration in feed was 0.0080%, and during the 2nd week, it was 0.0070%. These dietary concentrations were equal to a daily dose of approximately 6.5 mg/kg bw for both male and female animals. The rats were then switched to a diet containing [^{14}C]lasalocid sodium at a concentration of 0.0080% for 3 days (equal to 6.2 ± 0.3 mg/kg bw for male rats and 6.9 ± 0.7 mg/kg bw for female rats). Rats were killed 0, 1, 3 (five males and five females per time point) and 5 days (six males and six females) after withdrawal of the medicated diet (no information is provided on the fate of the remaining 10 rats). The livers from all males at each time point were combined, and the livers from all females at each time point were combined. Each pool of livers was homogenized and fractionated into ethanol-soluble and ethanol-insoluble fractions. The ethanol-insoluble fraction was subjected to base hydrolysis and partitioned between water and ethyl acetate.

Five days after withdrawal of the [^{14}C]lasalocid sodium diet, radioactivity was present in the livers of both sexes. In male rats, radioactivity was present at higher levels in the ethanol-soluble fraction than in the ethanol-insoluble fraction at days 0 and 1 after withdrawal of the diet, whereas on days 3 and 5, levels in

the ethanol-insoluble fraction exceeded those in the ethanol-soluble fraction. In female rats, levels in the ethanol-soluble fraction exceeded those in the ethanol-insoluble fraction at all time points. In the female rats on day 0, radioactivity in the insoluble fraction was predominantly in the ethyl acetate fraction (approximately 70%), whereas in males, the reverse was true. At subsequent time points, radioactivity was generally present to a greater degree in the water fraction in both sexes.

This study was performed with a view to providing a comparison with results previously seen in female chickens (Laurencot et al., undated). In that study, 16 chickens were pretreated with unlabelled lasalocid sodium in feed at a concentration of 0.0075% for 16 days. Twelve of 16 birds then received one oral capsule containing 5.0 mg of labelled drug per day for 3 days. The remaining four birds served as controls. Whole blood, fat, kidney, liver, muscle, skin, urine and faeces were collected, and radioactivity was determined by liquid scintillation counting. Radioactivity levels were highest in liver and decreased in the order liver > kidney > fat > skin > muscle. In liver, radioactivity was highest in the ethanol-soluble fraction at 2 hours and 24 hours after the last administration, but at subsequent time points (i.e. up to 5 days after the last administration), radioactivity levels were higher in the ethanol-insoluble fraction. Ninety-five per cent of the radioactivity was excreted in urine and faeces by 24 hours after the last dose. Comparison with the rat study indicates that in male rats and in chickens, there is a transition of residues in liver over time from alcohol-soluble forms to alcohol-insoluble forms. Differences in the alcohol and water solubility of residues seen in male and female rats indicate increased hydrophilicity of residues in males, which may be related to the fact that, in the toxicity studies, females typically displayed increased sensitivity compared with males (Anonymous, ca 1980).

In a study reported to have been performed in compliance with GLP (a signed quality assurance statement was provided), 10 rats (5 males and 5 females; strain not reported) were administered [^{14}C]lasalocid sodium as single oral (gavage) daily doses of approximately 1 mg/kg bw for 7 days. The drug was administered in 30% aqueous ethanol. Six additional rats (three males and three females) served as untreated controls. Urine and cage wash were collected at 24-hour intervals for each cage during treatment and up to 4 hours following the last dose. Faeces were collected from each cage at 24-hour intervals over the 7-day period. At 4 hours after the last dose, rats were killed. Radioactivity was determined in urine, cage wash, faeces and liver by liquid scintillation counting.

After the first oral dose, 67.37% of the dose was excreted within 24 hours, with 66.7% in faeces and 0.67% in urine. By 4 hours after the last dose, 73.46% of the dose was excreted in faeces and 0.6% in urine. No significant radioactivity was seen in cage wash (Hawkins, Elsom & de-Salis, 1987b).

Samples of pooled lyophilized livers and faeces were kept for metabolic profiling, which was undertaken as part of a separate study (Laurencot & Weiss, 1987) reported in section 2.1.2 below.

In a study that pre-dated GLP implementation, 10 adult Charles River CD rats (5 males and 5 females) were administered a single oral (gavage) dose of [^{14}C]lasalocid sodium at 1 mg/kg bw in 10% ethanol. Urine and faeces were collected (separately) from individual animals over the following intervals: 0–4 hours, 4–8 hours, 8–12 hours, 12–24 hours, 24–48 hours and 48–72 hours. Radioactivity was determined using liquid scintillation counting.

In male rats, approximately 92% of the administered radioactivity had been excreted in faeces by 72 hours, with approximately 80% excreted within the first 24 hours. Peak radioactivity levels were seen in faeces collected between 12 and 24 hours after administration.

In female rats, approximately 86% of the administered radioactivity had been excreted in faeces by 72 hours, with approximately 59% excreted within the first 24 hours. Peak radioactivity levels were seen in faeces collected between 12 and 24 hours after administration.

In both males and females, radioactivity in urine (over the entire 72-hour period) accounted for less than 1% of the administered radioactivity (Westheimer & Hutchinson, 1978b).

In a study that pre-dated GLP implementation, five adult male Charles River CD rats had gastric catheters surgically inserted and their bile ducts cannulated. The rats were infused intragastrically with a solution of sodium taurocholate in physiological saline to ensure hydration and steady-state conditions of bile flow. After a 24-hour recovery period, rats were administered a single dose of [^{14}C]lasalocid sodium via the gastric cannula at 1 mg/kg bw.

Bile was collected separately for each rat at 6–24 hours predosing and at 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–30 and 30–48 hours after dosing. Urine and faeces were also collected as separate samples for each animal at 0–24 hours predosing and at 0–4, 4–8, 8–12, 12–24 and 24–48 hours after dosing. At 48 hours after the last dose, the rats were killed and radioactivity was assessed in gastrointestinal tract contents, gastrointestinal tract tissue, liver and the remaining carcass. Radioactivity was assessed in tissues and excreta using liquid scintillation counting.

Approximately 59% of administered radioactivity was recovered in bile during the 48 hours following administration. Radioactivity levels were fairly constant in the bile samples collected at 0–2, 2–4, 4–6, 6–8, 8–12 and 12–24 hours (between 7% and 11% of the administered radioactivity in each) and declined to 1–2% in each of the intervals from 24 to 30 hours and from 30 to 48 hours. Approximately 37% of the administered radioactivity was recovered in faeces over the 48 hours after administration, with a further 1% in urine. At 48 hours after administration, approximately 1% of the administered radioactivity remained in liver, approximately 0.5% in gastrointestinal tract contents and less than 0.1% in gastrointestinal tract tissue; levels were below the level of detection in the remaining carcass.

The total absorbed dose (i.e. the radioactivity seen in bile, urine, liver and gastrointestinal tract tissue) accounted for an average of approximately 61% of the administered dose, almost all of which was excreted via the bile (59% of the

administered dose). The unabsorbed dose (i.e. the radioactivity seen in faeces and the gastrointestinal tract contents) accounted for an average of approximately 37% of the administered dose. In total, approximately 98% of the administered radioactivity was recovered (Laurencot et al., 1978).

2.1.2 Biotransformation

A comparative metabolism study (GLP status not stated) was performed in which metabolite profiles were examined in lyophilized faeces and livers resulting from oral administration of [^{14}C]lasalocid sodium to mouse, rat, dog, turkey, swine and chicken. The study used faeces and livers sourced from other studies and so does not provide information on the doses administered to the test animals or the dosing regimens, although the report numbers for the studies from which the livers and faeces were sourced are provided. The mouse and rat faeces and livers were generated in the studies reported in Hawkins, Elsom & de-Salis (1987a, 1987b), both of which used seven daily lasalocid doses of 1 mg/kg bw. The reports referenced for the studies from which the livers and faeces of other species were sourced were not provided.

Lyophilized liver samples were extracted with methanol, which was then diluted with aqueous sodium chloride and extracted with hexane. The hexane extract was analysed by high-performance liquid chromatography (HPLC) with radioactivity detection. The remaining aqueous methanol fraction was further diluted with aqueous sodium chloride before undergoing extraction with chloroform followed by separation using preparative thin-layer chromatography and then resolution using HPLC with radioactivity detection.

Intact lasalocid in the liver accounted for 6.2% of the radioactivity in pig liver, 3.8% in turkey liver, 11.4% in chicken liver, 18.1% in dog liver, 31.9% in rat liver and 28.1% in mouse liver.

For dog, rat, mouse, chicken and turkey, the proportion of the total radioactive residues (TRR) in liver extracted into methanol was 67.8%, 84.6%, 75.8%, 50.3% and 47.5%, respectively. The proportion of the TRR extracted into the hexane fraction was 21.0%, 35.3%, 28.7%, 26.3% and 5.6% for dog, rat, mouse, chicken and turkey, respectively. The proportion of the TRR extracted into the chloroform fraction was 47.6%, 32.9%, 37.8%, 28.0% and 36.3% for dog, rat, mouse, chicken and turkey, respectively.

In the HPLC analysis of the hexane extract, lasalocid was the major peak in all species (11.2%, 25.4%, 23.1%, 8.8% and 11.2% of the TRR in liver from the dog, rat, mouse, chicken and turkey, respectively). All other peaks represented less than 5% of the TRR in liver in all species. In the HPLC analysis of the chloroform extract, all peaks, including lasalocid, represented less than 10% of the TRR in liver, and all non-lasalocid peaks represented less than 5% of the TRR in liver.

Lyophilized faecal samples were extracted and fractionated in the same way as the liver samples.

Intact lasalocid in faeces accounted for 33.2% of the radioactivity in pig faeces, 32.2% in dog faeces, 43.7% in rat faeces, 10% in turkey faeces and 12% in chicken faeces.

For dog, rat, mouse, chicken and turkey, the proportion of the TRR extracted into methanol was 79.3%, 82.1%, 72.2%, 67.8% and 77.6%, respectively. The proportion of the TRR in faeces extracted into the hexane fraction was 42.3%, 68.6%, 30.2%, 17.% and 28.4% for dog, rat, mouse, chicken and turkey, respectively. The proportion of the TRR extracted into the chloroform fraction was 21.1%, 10.2%, 36.3%, 44.7% and 43.5% for dog, rat, mouse, chicken and turkey, respectively.

In the HPLC analysis of the hexane extract, lasalocid was the major peak in all species (32.0%, 43.4%, 21.0%, 11.6% and 9.7% of the TRR in faeces from the dog, rat, mouse, chicken and turkey, respectively). All other peaks represented less than 10% of the TRR in faeces in these species. In the HPLC analysis of the chloroform extract, all peaks, including lasalocid, represented less than 1% of the TRR in faeces.

This study supports the position that lasalocid is the main residue present in liver and faeces. Although the other residues have not been identified, they are considered to be minor (Laurencot & Weiss, 1987).

2.2 Toxicological studies

2.2.1 Acute toxicity

(a) Oral administration

In a study that pre-dated GLP requirements, mice, rats and rabbits were administered lasalocid sodium as single doses by several routes. Neonatal rats were also tested, and a single dog was administered increasing doses of the substance. Lasalocid sodium was ground in a mortar and suspended in 5% gum acacia solution. Animals were observed for 5 days following administration of the test substance.

Groups of 10 CF-1s mice were administered lasalocid sodium by the oral (gavage) route at a dose of 125, 160 or 200 mg/kg bw, by the intraperitoneal route at a dose of 50, 63, 80 or 100 mg/kg bw or by the subcutaneous route at a dose of 100, 125, 160 or 200 mg/kg bw. Mice of both sexes were used, although the ratio of male to female animals was not specified.

Groups of 10 Wistar rats were administered lasalocid sodium by the oral (gavage) route at a dose of 80, 100, 160 or 200 mg/kg bw or by the intraperitoneal route at a dose of 15, 25 or 50 mg/kg bw. Rats of both sexes were used, although the ratio of male to female animals was not specified.

Groups of four albino rabbits were administered lasalocid sodium by the oral (gavage) route at a dose of 25, 36, 45 or 50 mg/kg bw.

Groups of 10 neonatal (< 24 hours old) rats were administered lasalocid sodium by the oral (gavage) route at a dose of 20, 32 or 40 mg/kg bw. Rats of both sexes were used, although the ratio of male to female animals was not specified.

A single female dog was administered daily doses of lasalocid sodium at increasing daily doses (the dose was doubled every day, with a break in dosing over the weekend). The starting dose was 2.5 mg/kg bw, and the highest dose

administered was 160 mg/kg bw. The substance was administered in gelatine capsules. Plasma glucose and cholesterol were determined predosing and at 1 hour after each dose, as well as 6 days after the final dose. Blood counts, haemoglobin, haematocrit and blood enzymes were determined predosing and 1 hour after the 40, 80 and 160 mg/kg bw doses.

In mice, deaths were observed at all doses and by all routes, with the number of deaths increasing in a dose-dependent manner. The median lethal dose (LD_{50}) was calculated to be 146 mg/kg bw for the oral route, 68 mg/kg bw for the intraperitoneal route and 140 mg/kg bw for the subcutaneous route. The only other sign of toxicity observed was tremors, noted only in the intraperitoneally dosed mice.

In rats, deaths were observed at all doses in the orally dosed animals and at the top two doses in the intraperitoneally dosed animals. The number of deaths increased in a dose-dependent manner in both the orally and intraperitoneally dosed animals. The LD_{50} was calculated as 122 mg/kg bw for the oral route and 26.5 mg/kg bw for the intraperitoneal route. Other observed signs of toxicity reported were cyanosis and respiratory depression in the orally dosed animals and cyanosis, respiratory depression and decreased motor activity in the intraperitoneally dosed animals.

In rabbits, deaths were observed at all except the second lowest dose, with the highest number of deaths seen at the highest dose. The oral LD_{50} was calculated to be 40 mg/kg bw. No other signs of toxicity were reported.

In neonatal rats, a number of treated animals were missing and presumed to have been cannibalized and so counted as having died. A single death was observed at the low dose, no deaths at the middle dose and six deaths at the high dose. The oral LD_{50} was calculated to be 33 mg/kg bw.

In the dog study, no symptoms of toxicity were observed at 2.5 or 5 mg/kg bw. Clinical signs of toxicity were seen at 10 mg/kg bw and above. At 10 mg/kg bw, only emesis was seen. At higher doses, clinical signs included partial paralysis of hind quarters (at ≥ 20 mg/kg bw), wobbly gait and loss of appetite (at ≥ 40 mg/kg bw), defecation, salivation and severe ataxia (at 160 mg/kg bw). The dog survived to the end of the 6-day post-dosing monitoring phase. Haematology results showed increased white blood cell counts (from 7300 to 15 300 per cubic millimetre) 1 hour after the 160 mg/kg bw dose, which returned to predosing levels in the 6-day post-dosing blood sample. Small changes were seen in a number of the blood biochemistry parameters; however, given that a single animal was sampled, it is not possible to comment on the significance of these.

This study is incompletely reported, and the post-dosing observation period in the rodent and rabbit studies was limited to 5 days (Pool, Hane & Suckow, 1972).

The oral LD_{50} values above suggest that there are marked species differences, with the rabbit being significantly more sensitive than the rat or mouse and with neonatal rats being more sensitive than adult rats. This is consistent with published literature, which reports considerable species differences in susceptibility

to lasalocid toxicity. Horses have been reported to be particularly more sensitive to lasalocid toxicity than any of the above species, with an oral LD_{50} of 21.5 mg/kg bw estimated by one group (Hanson, Eisenbeis & Givens, 1981), although it has been argued that the real oral LD_{50} may be considerably lower in this species (Kronfeld, 2002). In addition, neonatal bovine calves have been reported to be more sensitive to lasalocid toxicity than older calves (Benson et al., 1998). A review of the acute toxicity of lasalocid is provided by Galitzer (1984).

(b) Dermal application

A pre-GLP study was performed in rabbits to investigate acute dermal toxicity. Two New Zealand White rabbits of each sex were administered single dermal doses of lasalocid sodium at 500, 1000 or 2000 mg/kg bw. The hair was clipped prior to administration of the substance, and the skin of one animal of each sex was abraded. The substance was applied as a powder under a gauze patch, and a plastic lined sleeve was placed over the trunk of each rabbit and fastened in place. After 24 hours, the wrappings were removed. Test animals were observed for 14 days in total, and deaths were recorded.

Signs of toxicity reported were decreased motor activity, cowering in the cage and lacrimation. All animals in the 500 mg/kg bw group survived. In the 1000 mg/kg bw group, one animal with abraded skin died. In the 2000 mg/kg bw group, three animals died (one with abraded skin and two with intact skin). A dermal LD_{50} of 1400 mg/kg bw was calculated (Hane, 1977).

(c) Dermal and ocular irritation

A pre-GLP study was performed in rabbits to investigate skin and eye irritation.

For skin irritation testing, three New Zealand White rabbits were used (it was reported that rabbits of both sexes were used, although the numbers of each sex were not reported). The hair was clipped from the back of each animal, and 500 mg of powder moistened with distilled water was applied to intact and abraded sites on each animal. The test sites were covered first with gauze patches and then with plastic occlusive coverings. After 4 hours, the coverings and patches were removed, and irritation was evaluated. Further irritation readings were taken after 24 and 48 hours. No signs of irritation were reported in any of the tested animals (Hane, 1977).

For ocular irritation testing, three New Zealand White rabbits of each sex were used. Lasalocid powder (0.036 g) was instilled into the conjunctival sac of one eye of each of six rabbits. The eyes of three rabbits were washed with water 5 minutes after instillation. The eyes of the remaining three rabbits were washed after 24 hours. Ocular reactions were graded 14 days after instillation. Signs of corneal irritation, conjunctival redness and chemosis were seen in both groups in which the eyes had been washed 5 minutes and 24 hours after instillation (Hane, 1977).

(d) Dermal sensitization

A pre-GLP study was performed to investigate the skin sensitizing potential of lasalocid sodium in the guinea-pig maximization test following both an intradermal and a topical induction phase. Twenty female albino guinea-pigs per group were divided into two groups of 10. The test group received intradermal injections of 0.1 mL of Freund's complete adjuvant, 0.1 mL of liquid petrolatum containing 1% lasalocid sodium and 0.1 mL of 1% lasalocid sodium emulsified in Freund's complete adjuvant. Control animals received the same series of injections with only vehicles and not lasalocid sodium. One week later, test guinea-pigs received a topical application of lasalocid sodium dispersed in liquid petrolatum at a concentration of 25%, applied over the injection site on filter paper and wrapped in an occlusive cover for 48 hours. Control animals received a topical application of liquid petrolatum. Two weeks after the initial topical application, all guinea-pigs (test and controls) were challenged by topical application of 25% lasalocid sodium in 0.5 mL of liquid petrolatum, applied on filter paper and wrapped in an occlusive cover for 24 hours. Twenty-four hours after application, the coverings were removed, and the application sites were washed with water. Erythema and oedema were scored on a scale of 0–4, and areas involved in irritant responses were measured. Evaluation took place 24 and 48 hours after challenge.

Erythema was observed in both test and control animals, without notable differences between those animals that underwent induction and those animals that did not. No oedema was noted in either group. It is concluded that lasalocid sodium did not induce skin sensitization in the guinea-pig maximization test (Hane et al., 1977).

2.2.2 Short-term studies of toxicity

(a) Rats

In a pre-GLP study, groups of 16 Charles River CD rats (8 males and 8 females) were administered lasalocid sodium in the diet at an intended dose of 0 (control), 2, 5 or 20 mg/kg bw per day for 13 weeks. Dosing at the desired level was maintained by adjusting the concentration in the diet on a weekly basis based on the average feed consumption and body weight of each group. Animals were individually caged and allowed feed ad libitum. Average actual drug intake levels were calculated for each sex at each dose level for each week. In males, average doses were 2.1 (range 1.4–3.3), 5.2 (range 4.3–6.7) and 20.3 (range 17.1–27.8) mg/kg bw per day in the low-, mid- and high-dose groups, respectively; in females, they were 2.3 (range 1.5–3.6), 5.2 (range 4.2–6.2) and 19.7 (range 16.0–24.0) mg/kg bw per day, respectively.

Animals were observed daily for physical and behavioural signs of toxicity. Feed consumption and body weights were measured weekly. Neurological examinations (observations of gait, body position, muscle tone, movement of legs and reflexes) were performed prior to treatment and after 4, 8 and 12 weeks. Ophthalmic examinations (observations of eyelids, conjunctiva and sclera, examination of cornea, iris, lens and fundus with an ophthalmoscope) were performed at the onset of treatment, after 4 and 8 weeks and during the

12th week. Haematological examinations were performed on five rats of each sex per group prior to treatment and after 4, 8 and 12 weeks. Haematological examinations included measurement of the susceptibility of erythrocytes to haemolysis in hypotonic saline following collection of blood at necropsy. This was done because it was noted, while performing haemoglobin analyses, that some red blood cell samples required longer than expected to lyse. Heparinized whole blood was mixed with hypotonic saline (0.35%, 0.40%, 0.45% and 0.50% saline), and, after 30 minutes, intact cells were separated by centrifugation. Haemolysis was estimated in the supernatant by measuring light transmission. The per cent haemolysis was reported by comparison with measurements taken following mixing of whole blood with physiological saline. These measurements were also performed with blood from additional rats administered lasalocid sodium intravenously as a single dose of 5 mg/kg bw. Blood glucose was determined in samples from five rats of each sex per group after 4, 8 and 12 weeks. Blood biochemistry parameters were determined in samples collected at termination. Urine analysis was undertaken on urine collected individually from five rats of each sex per group prior to treatment and after 4, 8 and 12 weeks. At necropsy, organs were examined grossly, weighed and prepared for microscopic evaluation.

No compound-related mortality occurred during the study. Feed consumption and body weights were significantly reduced in females in the 20 mg/kg bw per day group over the entire dosing period (by week 13, body weights of females were 62% of those in the control group). The overall pattern of feed consumption and body weight in males in the 20 mg/kg bw per day group did not differ from that seen in control males, although average weekly feed intake and body weights were statistically significantly different between the two groups at a number of discrete time points. Feed consumption and body weights were reduced in females in the 5 mg/kg bw per day group compared with control females, although the effect did not reach statistical significance. No effects on feed consumption or body weight were seen in males in the 5 mg/kg bw per day group or in either sex in the 2 mg/kg bw per day group. No compound-related behavioural or neurological signs of toxicity were seen.

Haemoglobin and haematocrit levels were slightly (but statistically significantly) decreased in females at 20 mg/kg bw per day at the 4-week time point only. At other time points, haemoglobin, haematocrit, coagulation times and differential white blood cell counts showed only minor and transient variations across the dose groups, which were not considered to be drug related. In males and females in the 20 mg/kg bw per day group, mild variations in erythrocyte size and shape were noted, as well as some polychromatic and crenated erythrocytes and target cells. Investigations of the susceptibility of erythrocytes to haemolysis in hypotonic saline demonstrated reduced haemolysis in blood of female rats administered lasalocid sodium (e.g. after mixing with 0.40% saline, 92% haemolysis was seen for blood from control rats, compared with 68%, 44% and 29% for blood from female rats administered lasalocid sodium at 2, 5 and 20 mg/kg bw per day, respectively). An effect was also seen in male rats, although it was less marked, and the dose–response relationship was less clear (e.g. after mixing with 0.40% saline, 77% haemolysis was seen for blood of control rats, compared with 78%, 74% and 60% for blood from male rats administered lasalocid sodium at

2, 5 and 20 mg/kg bw per day, respectively). Haemolysis was also reduced in blood from male and female rats treated with a single intravenous dose of 5 mg/kg bw. The study authors argued that this effect should not be considered as adverse.

Blood glucose levels in males were unaffected. At 12 weeks after the start of dosing, blood glucose levels were statistically significantly reduced in female rats administered 2 and 20 mg/kg bw per day, but not 5 mg/kg bw per day. At 8 weeks after the start of dosing, blood glucose levels were statistically significantly reduced in female rats administered 20 mg/kg bw per day only. The effect was slight and not dose related and is not considered to be of biological significance. A statistically significant increase in serum alkaline phosphatase (AP) was seen in males and females in the 20 mg/kg bw per day group. Alanine aminotransferase (ALAT) levels were statistically significantly reduced in males in the 5 mg/kg bw per day group only and were statistically significantly decreased in females in the 5 mg/kg bw per day group and statistically significantly increased in females in the 20 mg/kg bw per day group. Aspartate aminotransferase (ASAT) levels were statistically significantly increased in males and females in the 20 mg/kg bw per day group. Blood urea nitrogen levels were statistically significantly decreased in males in the 20 mg/kg bw per day group. For many animals, the volume of serum available was too low to allow measurement of serum sodium, potassium, chloride and calcium levels. No clear pattern emerges from the available measurements.

There were no findings of note in the urine analysis.

The only lesion noted at gross pathology evaluation was an enlarged uterus in one rat in the 5 mg/kg bw per day group.

In females in the 20 mg/kg bw per day group, absolute lung, liver, kidney, adrenal gland, pituitary gland and ovary weights were decreased and relative (to body weight) heart, lung, liver, kidney, thyroid, adrenal, pituitary gland, spleen and brain weights were significantly increased. Ovary to body weight ratio was statistically significantly decreased.

In females at 5 mg/kg bw per day, liver to body weight ratios were increased.

In females in the 2 mg/kg bw per day group, uterus weights were statistically significantly decreased overall, although the organ weight was substantially increased in a single animal; uterus to body weight ratios were statistically significantly decreased. In the 5 mg/kg bw per day group, uterus weights were statistically significantly increased; uterus to body weight ratios were statistically significantly increased. In the 20 mg/kg bw per day group, uterus weights were statistically significantly decreased; uterus to body weight ratios were statistically significantly decreased. Whereas a decrease in uterus weight was seen in six of eight animals in the 20 mg/kg bw per day group, the effect in the 2 and 5 mg/kg bw per day groups was less clear and consistent. Based on the lack of a dose–response relationship and the absence of histopathological findings at all doses, the effect in the 2 and 5 mg/kg bw per day groups is not considered biologically significant.

In males in the 2 mg/kg bw per day group, only pituitary weights were slightly decreased. No effect on the pituitary was seen at higher doses; consequently, this

effect was not considered biologically significant. In males in the 5 mg/kg bw per day group, only testes weights were slightly decreased. No effect was seen on the testes at the higher dose. Liver to body weight ratios were slightly decreased at 5 mg/kg bw per day. No effect was seen at the higher dose. Based on the absence of a dose–response relationship, these effects were not considered drug related. In males in the 20 mg/kg bw per day group, only brain weights were slightly decreased, and heart to body weight ratio was slightly decreased. No effect on brain or heart weight was seen at lower doses.

Histopathological evaluation revealed increased haemosiderin in liver (Kupffer cells) and kidney epithelial cells in all females and in four and two males (livers and kidneys, respectively) at 20 mg/kg bw per day. Increased haemosiderin was also seen in the liver of a single female at 5 mg/kg bw per day. In six of eight females in the 20 mg/kg bw per day group, large vacuoles were noted in the pituitary. Small vacuoles in the myocardium were also noted in seven females and three males in the 20 mg/kg bw per day group, with similar vacuoles in skeletal muscle reported in five females in the 20 mg/kg bw per day group.

It is noted that the number of animals included in this study (eight of each sex per dose) was less than would be expected in a study performed for regulatory purposes today (Organisation for Economic Co-operation and Development [OECD] Test Guideline 408 specifies 10 of each sex per dose).

The only effect noted at the lowest dose of 2 mg/kg bw per day was a reduced susceptibility of erythrocytes to haemolysis in hypotonic saline. This effect is consistent with reports of studies performed with the divalent ionophore A23187. A number of authors (Reed & Lardy, 1972; White, 1974; Kuettner et al., 1977) have reported effects of A23187 in vitro, which has been observed to cause calcium-dependent loss of potassium, water and adenosine triphosphate from erythrocytes, as well as a reduced sensitivity to osmotic lysis. Kuettner et al. (1977) further reported that the increased calcium content of erythrocytes caused by A23187 induced morphological changes and reduced viscoelastic properties. Bogin et al. (1982) reported that A23187 reduced erythrocyte membrane fragility in the absence of calcium, but increased membrane fragility in the presence of calcium. It is notable that in the study under evaluation, effects on erythrocyte morphology were seen, although only at 20 mg/kg bw per day. The perturbation of ionic flux across the erythrocyte membrane induced by lasalocid has been specifically studied using nuclear magnetic resonance (Fernandez, Grandjean & Laszlo, 1987). It is not surprising that the ionophoretic effects of lasalocid should have an impact on (erythrocyte) membrane stability. The effect seen in this study was observed ex vivo and was not associated with a toxicological effect in vivo. Consequently, the increased resistance to lysis in hypotonic saline is not, in itself, considered to be an adverse effect.

The no-observed-adverse-effect level (NOAEL) was 2 mg/kg bw per day, based on reduced feed consumption, increased liver to body weight ratios and increased haemosiderin in the liver in females at 5 mg/kg bw per day (Pfitzer & Roberts, 1973).

In a pre-GLP study, groups of 80 Charles River CD weanling rats (40 males and 40 females) were administered lasalocid sodium in the diet at an intended dose of 0 (control), 1, 2, 3 or 10 mg/kg bw per day for 13 weeks. Dosing at the desired level was maintained by adjusting the concentration based on the average feed consumption and body weight of each group. Diets were adjusted for appropriate doses twice a week for the first 3 weeks (when feed consumption relative to body weight was rapidly decreasing) and weekly thereafter. Animals were caged individually and allowed feed ad libitum. Average actual drug intake levels were calculated for each sex at each dose level twice a week for the first 3 weeks and weekly thereafter. Average weekly group intakes (overall averages over the entire study period) were, for males, 0.72–1.11 (0.95), 1.35–2.56 (1.93), 2.25–3.34 (2.89) and 5.84–13.43 (9.70) mg/kg bw per day at 1, 2, 3 and 10 mg/kg bw per day, respectively; and for females, 0.66–1.21 (0.97), 1.44–2.54 (1.92), 1.79–3.84 (2.93) and 6.12–11.98 (9.39) mg/kg bw per day at 1, 2, 3 and 10 mg/kg bw per day, respectively.

Twenty male and 20 female rats from each group were killed after 8 weeks, and the remainder were killed at the end of 13 weeks. Animals were observed daily for physical and behavioural signs of toxicity. Feed consumption and body weights were measured weekly. Neurological examinations (observations of gait, body position, muscle tone, movement of legs and reflexes) were performed prior to treatment and after 4, 8 and 12 weeks. Ophthalmic examinations (observations of eyelids, conjunctiva and sclera, examination of cornea, iris, lens and fundus with an ophthalmoscope) were performed after 1 week of treatment and prior to necropsy at 8 and 13 weeks. Haematological examinations were performed on blood samples taken after 7 and 12 weeks. Blood biochemistry parameters were determined in samples taken from animals killed at 8 and 13 weeks. Urine analysis was undertaken on urine collected from each rat after 7 and 12 weeks. At necropsy, organs were examined grossly, weighed and prepared for microscopic evaluation.

No compound-related mortality occurred during the study. Body weights were significantly reduced in females in the 10 mg/kg bw per day group from weeks 8 to 13 (by week 13, body weights of females were 88.5% of those in the control group). Feed consumption in females in the 10 mg/kg bw per day group was also slightly reduced from weeks 9 to 13, although the effect did not reach statistical significance. Body weights in males in the 10 mg/kg bw per day group were slightly decreased in the last weeks of the study, but the effect did not reach statistical significance. No effect on feed consumption was evident in males in the 10 mg/kg bw per day group. No compound-related effects on body weight or feed consumption were seen in either sex in the other groups. No compound-related behavioural or neurological signs of toxicity were seen. No compound-related ophthalmic findings were noted.

Compared with controls, haematocrit and haemoglobin levels were statistically significantly decreased after week 7 in female rats in the 2, 3 and 10 mg/kg bw per day groups and after week 12 in female rats in the 3 and 10 mg/kg bw per day groups; haematocrit (but not haemoglobin) was statistically significantly decreased in females in the 2 mg/kg bw per day group at week 12. In male rats, haematocrit and haemoglobin were statistically significantly decreased

after both weeks 7 and 12 in the 1, 3 and 10 mg/kg bw per day groups but not in the 2 mg/kg bw per day group. Based on the lack of a dose–response relationship and on the small magnitude of the decreased haematocrit and haemoglobin levels seen in males in the 1 mg/kg bw per day group (96% or 97% of control values in all cases), the effect at this dose is not considered to be a drug effect. Coagulation times were slightly but statistically significantly reduced in both males and females in the 10 and 3 mg/kg bw per day groups after 12 weeks. A slight increase in coagulation time was seen in males in the 1 mg/kg bw per day group after 7 weeks, but was not considered to be drug related. Increased white blood cell counts were seen in females in the 10 mg/kg bw per day group after both 7 and 12 weeks, and increased white blood cell counts were seen in males in the 10 mg/kg bw per day group after 12 weeks only. Lymphocyte counts were statistically significantly decreased in females in the 10 mg/kg bw per day group and in females in the 3 mg/kg bw per day group at 12 weeks only. Neutrophil counts were statistically significantly increased in females in the 2, 3 and 10 mg/kg bw per day groups after both 7 and 12 weeks. In males, neutrophil counts were increased in the 3 and 10 mg/kg bw per day groups, but the effect did not reach statistical significance. Eosinophil counts were statistically significantly increased in females in the 2 and 3 mg/kg bw per day groups (but not in the 10 mg/kg bw per day group) after 12 weeks. The study report notes that morphological changes (target cells) were seen in a minority of erythrocytes after 12 weeks in all female rats in the 10 mg/kg bw per day group and in 7/20 females in the 3 mg/kg bw per day group; the finding was also noted in a small number of males at these doses. Small numbers of nucleated erythrocytes were noted in 3/20 females in the 10 mg/kg bw per day group and in 1/20 females in the 3 mg/kg bw per day group. Unlike in the previous study (Pfitzer & Roberts, 1973), susceptibility of erythrocytes to haemolysis was not specifically measured in this study. However, the study report indicates that in the haemoglobin analysis, increased resistance to lysis was noted at the 10 mg/kg bw per day dose in all females and in a number of males.

AP was statistically significantly increased in male rats in all dose groups at 13 weeks and in the 2 and 10 (but not the 1 and 3) mg/kg bw per day groups at 8 weeks. AP was statistically significantly increased in female rats in the 2, 3 and 10 mg/kg bw per day groups at both 8 and 12 weeks. The study authors argued that AP decreases markedly with age from around 400–500 International Units (IU) at weaning and that, consequently, the increased AP seen in this study represents a reduced decrease relative to controls (rather than an absolute increase). They further argued that, in this study, AP decreased particularly rapidly in the control group (control group averages at 8 and 13 weeks were reported to be 211.8 and 144.9 IU in males and 134.1 and 103.3 IU in females, respectively), whereas in a concurrent study using weanling rats, the values decreased less rapidly (control group averages at 8 and 13 weeks were reported to be 261.5 and 222.4 IU in males and 151.9 and 146.1 IU in females, respectively); on the basis of this information, the only biologically significant effect on AP was considered to occur in females in the 10 mg/kg bw per day group. This argument is not accepted. In the concurrent study, rats were treated with the same doses of lasalocid sodium, and measurements were taken at similar time points. However, it is noteworthy that the AP values reported in that study are higher in most dose groups, not only in the

control groups (suggesting that it is not appropriate to compare the control data from one study with the test data from the other study), although the difference in the values reported for the control groups in the two studies is a little more marked than in most other groups.

ASAT levels were statistically significantly increased in females in the 10 mg/kg bw per day group after 13 weeks only, and blood urea nitrogen levels were statistically significantly decreased in females in the 10 mg/kg bw per day group after 13 weeks and in males in the 3 and 10 mg/kg bw per day groups at 13 weeks.

Serum calcium levels were statistically significantly elevated in females at all doses at 8 weeks only and in males in the 2, 3 and 10 mg/kg bw per day groups at 8 weeks only. Serum sodium levels were statistically significantly increased in females in all dose groups at 8 weeks and in the 3 mg/kg bw per day group at both 8 and 13 weeks. In males, serum sodium levels were statistically significantly increased in the 2, 3 and 10 mg/kg bw per day groups at 8 weeks and in the 3 mg/kg bw per day group at 13 weeks also. Serum potassium levels were increased in females in the 10 mg/kg bw per day group at 8 weeks only and in males in the 3 and 10 mg/kg bw per day groups at 8 weeks only. Serum chloride levels were increased in males and females in the 10 mg/kg bw per day group at 13 weeks only. It is noteworthy that all changes in electrolyte levels seen in the 1, 2 and 3 mg/kg bw per day groups were within a few per cent of control values (the largest deviation was a value of 106% for calcium seen in females at 3 mg/kg bw per day at 8 weeks). Although the changes reached statistical significance on a number of occasions, whether or not the changes were drug related is questionable. At 10 mg/kg bw per day, some slightly larger variations were noted (the largest deviation from control was for serum potassium and calcium levels, which were both at 112% of control levels in females at 8 weeks).

There were no findings of note in the urine analysis.

No lesions considered to be compound related were noted at gross pathology.

In females, the following statistically significant organ weight changes were noted at 10 mg/kg bw per day: decreased absolute heart weight at week 13, decreased absolute and relative (to body weight) lung weights at weeks 8 and 13, increased relative liver weight at 13 weeks, increased relative kidney weight at weeks 8 and 13, decreased absolute ovary weight at week 8 and decreased absolute and relative ovary weights at week 13, increased relative thyroid weight at weeks 8 and 13, increased absolute adrenal weight at week 8, increased relative pituitary weight at week 8 and increased relative brain weight at week 8. At 3 mg/kg bw per day, the following statistically significant organ weight changes were noted: increased relative heart weight at week 8, decreased relative lung weight at week 13, increased relative liver weight at week 13, increased relative thyroid weight at week 13 and increased relative pituitary weight at weeks 8 and 13. In females in the 1 and 2 mg/kg bw per day groups, relative pituitary weights were statistically significantly increased at 13 weeks. Although relative kidney weights were not statistically different from controls in the 3 mg/kg bw per day group, they were statistically significantly different from controls in the 1 and 2 mg/kg bw per day groups at 8 weeks only.

In males, the following statistically significant organ weight changes were noted at 10 mg/kg bw per day: decreased absolute and relative heart weights at weeks 8 and 13 and decreased absolute and relative lung weights at week 13; at 3 mg/kg bw per day: decreased absolute and relative heart weights at week 13 and decreased relative heart weight at week 8; at 2 mg/kg bw per day: decreased absolute and relative heart weights at week 8; and at 1 mg/kg bw per day: decreased relative heart weight at week 8. A number of other statistically significant organ weight changes were seen, but were not considered compound related, as they occurred with no particular pattern.

A full histopathological examination was performed on five male and five female rats in the 10 mg/kg bw per day group and control group killed after 8 weeks. Histopathological evaluations focusing on liver, kidney and heart were performed on 15 additional male and female rats from the 10 mg/kg bw per day group and the control group as well as on 20 male and 19 female rats in the 3 mg/kg bw per day group. There was no histopathological examination of rats from the 1 and 2 mg/kg bw per day groups killed after 8 weeks.

After 8 weeks, vacuoles were noted in the myocardial fibres of females administered 10 mg/kg bw per day. The vacuoles were not stained by eosin or fat stains. The effect was not seen in animals administered 3 mg/kg bw per day. In the liver, haemosiderin was seen in Kupffer cells in the 3 and 10 mg/kg bw per day groups, with the effect being more pronounced at the higher dose. In the kidney, haemosiderin was moderately increased in some females administered 10 mg/kg bw per day. No differences compared with controls were noted in the 3 mg/kg bw per day group.

A full histopathological examination was performed on 20 male and 20 female rats in the 10 mg/kg bw per day group and control group killed after 13 weeks. Histopathological evaluations focusing on liver, kidney and heart were performed on 20 male and 20 female rats in the 2 and 3 mg/kg bw per day groups. There was no histopathological examination of rats from the 1 mg/kg bw per day group.

After 13 weeks, vacuoles were noted in the myocardial fibres of females administered 10 mg/kg bw per day. The vacuoles were not stained by eosin or fat stains. The effect was not seen in animals administered 2 or 3 mg/kg bw per day. In the liver, increased (relative to control) haemosiderin was seen in Kupffer cells in females and males in the 10 mg/kg bw per day group and in females only at 3 mg/kg bw per day, with the effect being more pronounced at the higher dose. In the kidney, haemosiderin was increased in some females in the 3 and 10 mg/kg bw per day dose groups, with the effect being more common at the higher dose. No differences compared with controls were noted in the 2 mg/kg bw per day group.

Based on increased AP levels seen in males at all doses at week 13, the lowest-observed-adverse-effect level (LOAEL) for this study was 1 mg/kg bw per day. No NOAEL was established (Pfitzer & Roberts, 1975a). It is noted, however, that the low-dose effect on AP seen in this study was not seen in other rat studies.

In a pre-GLP study, groups of 120 Charles River CD weanling rats (60 males and 60 females) were administered lasalocid sodium in the diet at an intended dose of 0 (control), 1, 2, 3 or 10 mg/kg bw per day for 13 weeks. The weanling rats were selected from offspring of rats that had been administered the same intended dose of lasalocid sodium in the diet. Parental animals were dosed for 3 weeks prior to mating and during mating (approximately 2 weeks), gestation and lactation (approximately 6 weeks). Weanling rats received lasalocid sodium in the diet immediately upon weaning and for 13 weeks thereafter. The present study (Pfitzer & Roberts, 1975b) detailed only the effects on weanling rats. In relation to effects on the parental generation, it reported that no effects were seen on the various parameters of fertility and reproductive performance except at the 10 mg/kg bw per day dose, at which there were reduced maternal and neonatal weight gains. The effects on reproductive parameters of the parental animals are reported in Hoar et al. (1974) and described in section 2.2.5 below.

Dosing of weanlings at the desired level was maintained by adjusting the concentration based on the average feed consumption and body weight of each group. Diets were adjusted for appropriate doses on a weekly basis. Animals were caged individually and allowed feed ad libitum. Average actual drug intake levels were calculated for each sex at each dose level on a weekly basis. Average weekly group intakes (overall averages over the entire study period) were, for males, 0.85–1.30 (0.96), 1.69–2.99 (1.95), 2.57–4.45 (2.99) and 8.37–13.03 (9.60) mg/kg bw per day at 1, 2, 3 and 10 mg/kg bw per day, respectively; and, for females, 0.79–1.24 (0.96), 1.65–2.54 (1.94), 2.51–5.63 (3.09) and 8.50–13.29 (9.80) mg/kg bw per day at 1, 2, 3 and 10 mg/kg bw per day, respectively.

Twenty male and 20 female rats from each dose group were killed after 4, 8 and 13 weeks. Animals were observed daily for physical and behavioural signs of toxicity. Feed consumption and body weights were measured weekly. Ophthalmic examinations (observations of eyelids, conjunctiva and sclera, examination of cornea, iris, lens and fundus with an ophthalmoscope) were performed after 1 week of treatment on all rats, after 3 weeks on 20 animals of each sex per dose, after 8 weeks on 20 animals of each sex per dose and after 11 and 13 weeks on the remaining animals. Haematological examinations were performed after 2, 7 and 12 weeks on 20 rats of each sex per group. Blood biochemistry parameters were determined in samples taken from animals killed at 4, 8 and 13 weeks. Urine analysis was undertaken on urine collected from rats after 2, 7 and 12 weeks. At necropsy, organs were examined grossly, weighed and prepared for microscopic evaluation. Only tissues from animals killed at 4 and 13 weeks were examined histologically.

No compound-related mortality occurred during the study. At the start of the 13-week dosing period, body weights of female weanlings in the 10 mg/kg bw per day dose group were statistically significantly reduced (compared with concurrent controls), but these had recovered by the end of the 1st week. Body weights were statistically significantly reduced in females in the 10 mg/kg bw per day group in weeks 12 and 13 (by week 13, body weights of females were 91.9% of those in the control group). Feed consumption of females in the 10 mg/kg bw per day group was reduced in weeks 11, 12 and 13 (down to 82.6% of that seen

in control females at 13 weeks). Feed consumption was also reduced in females in the 2 and 3 mg/kg bw per day groups in weeks 12 and 13, although the effect reached statistical significance only in the 2 mg/kg bw per day group at week 12. Body weights of male test animals were not statistically significantly different from those of control animals. Feed consumption was statistically significantly reduced in males in the 3 and 10 mg/kg bw per day groups. Differences in mean body weights and feed consumption occurred between controls and treatment groups at a number of other time points; however, due to their isolated occurrence, these are not considered biologically significant. No compound-related behavioural signs of toxicity were seen. Ophthalmic examination revealed increased levels of focal retinal degeneration in the 3 and 10 mg/kg bw per day groups. Focal retinal degeneration was also seen in rats in the 1 and 2 mg/kg bw per day groups, but the incidence was similar to that seen in the control group. The sponsor argued that the condition occurs spontaneously in the laboratory rat, that it has a multifactorial etiology (possibly including bacterial or viral infection) and that the increased incidence seen in the 3 and 10 mg/kg bw per day groups is likely to be an indirect effect resulting from increased overall stress in these groups and not a direct effect of the drug.

Compared with controls, haematocrit levels were statistically significantly decreased in females in the 10 mg/kg bw per day group after weeks 7 and 12; haemoglobin levels were statistically significantly decreased after week 12 only. Haematocrit levels were also decreased in female rats in the 2 and 3 mg/kg bw per day groups after week 2 only. In male rats, haematocrit and haemoglobin levels were statistically significantly decreased in the 10 mg/kg bw per day group after 12 weeks; after 2 weeks, haematocrit levels were statistically significantly decreased in the 1, 2 and 3 mg/kg bw per day groups (no effect seen after 7 or 12 weeks). All changes in haematocrit and haemoglobin levels were slight; additionally, some sporadic increases in haemoglobin were seen. Overall, the effect at 10 mg/kg bw per day (seen in both haematocrit and haemoglobin in both sexes at week 12) is considered possibly compound related. Other changes were seen only at interim time points and only in either haematocrit or haemoglobin and are not considered compound related. No compound-related changes in coagulation time occurred. Unlike in the Pfitzer & Roberts (1973) study, susceptibility of erythrocytes to haemolysis was not specifically measured in this study. However, the study report indicates that in the haemoglobin analysis, increased resistance to lysis was noted at the 10 mg/kg bw per day dose, particularly in females.

Statistically significantly increased white blood cell counts were seen in males and females in the 10 mg/kg bw per day group after 2, 7 and 12 weeks. Statistically significantly increased white blood cell counts were also seen in females in the 3 mg/kg bw per day group after 2 and 7 (but not 12) weeks, in females in the 2 mg/kg bw per day group after 2 weeks only, in males in the 3 mg/kg bw per day group after 7 weeks only and in males in the 2 mg/kg bw per day group after 2 weeks only. Lymphocyte counts were statistically significantly increased in females in the 10 mg/kg bw per day group at 2 weeks only and in males in the 10 mg/kg bw per day group at 12 weeks only. Lymphocytes were also statistically significantly increased in females at 2 mg/kg bw per day (2 weeks only) and 3 mg/kg bw per day (2 and 7 weeks). Neutrophil counts were statistically

significantly decreased in females in the 2 mg/kg bw per day group (2 weeks only), 3 mg/kg bw per day group (weeks 2 and 7) and 10 mg/kg bw per day group (week 2 only). In males, neutrophil counts were statistically significantly decreased in the 10 mg/kg bw per day group at 12 weeks. All of the above changes were slight, and only the increased white blood cell count at 10 mg/kg bw per day occurred at all time points. The study report noted that morphological changes (target cells) were seen in a minority of erythrocytes at 10 mg/kg bw per day in both sexes, although predominantly in females, after 2, 7 and 12 weeks. The report indicated that similar findings were seen in isolated individual rats at other doses, but the frequency and specific doses were not given.

AP was statistically significantly increased in female rats in the 10 mg/kg bw per day group after 4, 8 and 13 weeks. In male rats in the 10 mg/kg bw per day group, AP was increased, but only after 4 and 8 weeks. AP was also increased in males and females at 3 mg/kg bw per day, but only at interim time points. ASAT levels were statistically significantly increased in females in the 10 mg/kg bw per day group after 8 and 13 weeks. Other clinical biochemistry results differed significantly from controls only sporadically and are not considered compound related.

There were no findings of note in the urine analysis.

No lesions considered to be compound related were noted at gross pathology.

In females, the following statistically significant organ weight changes were noted at 10 mg/kg bw per day: increased relative heart weight at week 13, increased relative lung weight at week 13, increased relative liver weight at weeks 4 and 13, increased relative kidney weight at weeks 4, 8 and 13, decreased absolute ovary weight at week 13, increased absolute and relative uterus weights at week 8 (relative uterus weight was also increased at week 13, but the effect did not reach statistical significance), increased absolute and relative spleen weights at week 8 and increased relative spleen weight at week 13, increased relative adrenal weight at week 13 and increased relative brain weight at week 13; relative pituitary weights were increased at week 4, decreased at week 8 (along with absolute weight) and increased at week 13. At 3 mg/kg bw per day, the following statistically significant organ weight changes were noted: increased absolute and relative lung, liver and kidney weights at week 4 only, increased absolute and relative ovary weights at week 4, increased absolute and relative uterus weights at week 8 only, increased absolute and relative adrenal weights at week 4 only and increased absolute pituitary weight at week 8 only. At 2 mg/kg bw per day, the following statistically significant organ weight changes were noted: increased absolute and relative liver weights at week 4 only, increased absolute and relative ovary weights at week 4 only and increased absolute and relative adrenal weights at week 13. At 1 mg/kg bw per day, the following statistically significant organ weight changes were noted: increased absolute and relative liver weights at week 4 only and increased absolute and relative ovary weights at week 4 only. A small number of additional statistically significant deviations from control values were seen; however, due to their sporadic nature, they are not considered compound related. Many of the above changes, although occurring consistently across dose groups, occurred only at interim time points. The only findings seen after 13 weeks and considered possibly compound

related are those seen in the 10 mg/kg bw per day group (increased heart, lung, liver, kidney, adrenal, pituitary and brain weights and decreased ovary weight).

In males, the following statistically significant organ weight changes were noted at 10 mg/kg bw per day: decreased absolute heart weight at week 4 only, decreased absolute lung weight at week 4 only, decreased absolute testis weight at week 4 only, increased relative adrenal weight at weeks 4 and 13, increased absolute and relative pituitary weights at week 8 and increased relative pituitary weight at week 13, and increased relative brain weight at week 13. At 3 mg/kg bw per day, the following statistically significant organ weight changes were noted: decreased absolute heart weight at week 13, increased relative liver weight at week 4 only, increased absolute and relative adrenal weights at week 4 only and increased relative pituitary weight at week 13. At 2 mg/kg bw per day, the following statistically significant organ weight changes were noted: increased relative liver weight at week 13, increased absolute and relative adrenal weights at week 4 only and increased absolute and relative pituitary weights at week 13. At 1 mg/kg bw per day, the following statistically significant organ weight changes were noted: increased relative liver weight at week 4 only, increased relative adrenal weight at week 4 only and increased absolute and relative pituitary weights at week 13. A small number of additional statistically significant deviations from control values were seen, but, due to their sporadic nature, are not considered compound related. Many of the above changes, although occurring consistently across dose groups, occurred only at interim time points. The only findings seen after 13 weeks and considered possibly compound related are the increased adrenal weights seen at 10 mg/kg bw per day and the increased pituitary weights seen at all doses.

A full histopathological examination was performed on 20 male and 20 female rats in the 10 mg/kg bw per day group and control group killed after 4 weeks. Histopathological evaluations focusing on heart only were performed on 20 male and 20 female rats from the 3 mg/kg bw per day group. There was no histopathological examination of rats from the 1 and 2 mg/kg bw per day groups killed after 4 weeks.

After 4 weeks, vacuoles were noted in the myocardial fibres of females administered 10 mg/kg bw per day. The vacuoles were not stained by eosin or fat stains. The effect was not seen in animals administered 3 mg/kg bw per day. In the eye, focal loss of the epithelial layer and the layers of rods and cones was seen in one male in the 10 mg/kg bw per day group and in six controls.

A full histopathological examination was performed on 20 male and 20 female rats in the 10 mg/kg bw per day group and control group killed after 13 weeks. Histopathological evaluations focusing on liver, kidney and heart were performed on 20 male and 20 female rats in the 1, 2 and 3 mg/kg bw per day groups.

After 13 weeks, vacuoles were noted in the myocardial fibres of 13/20 females and 1/20 males administered 10 mg/kg bw per day. The vacuoles were not stained by eosin or fat stains. The effect was not seen in animals administered 2 or 3 mg/kg bw per day. In the liver, increased (relative to control) haemosiderin was seen in Kupffer cells in 17 females and 2 males in the 10 mg/kg bw per day group and in 5 females and 1 male at 3 mg/kg bw per day. The report

indicates that haemosiderin was also seen at 2 mg/kg bw per day, but that the amount was consistent with that seen in control livers. In the kidney, haemosiderin was increased in eight females and one male in the 10 mg/kg bw per day dose groups. In the eye, focal retinal lesions were seen in two rats in the 10 mg/kg bw per day group and in three controls. In a number of rats in which retinal degeneration was noted in the ophthalmoscopy examination, no histological effect was identified.

Based on increased haemosiderin seen in the liver of males and (predominantly) females at a dose of 3 mg/kg bw per day, the NOAEL was 2 mg/kg bw per day. In the absence of histopathological correlates, the organ weight effects seen at the lowest dose (increased absolute and relative pituitary weights) are not considered to represent an adverse drug effect (Pfitzer & Roberts, 1975b).

(b) Dogs

In a pre-GLP study, groups of six Beagle dogs (three males and three females) were administered lasalocid sodium in gelatine capsules as a single daily dose of 2, 5 or 10 mg/kg bw per day for 13 weeks. A further group of three males and three females acted as controls. Controls did not receive a placebo. Animals were caged individually and allowed feed ad libitum.

Animals were observed daily for physical and behavioural signs of toxicity as well as feed consumption. Body weights were measured weekly. Ophthalmic and neurological examinations (observations of posture, gait and reflexes) were performed on each dog prior to treatment and at approximately monthly intervals. Ophthalmic examinations included observations of eyelids, conjunctiva and sclera and examination of cornea, lens and fundus with an ophthalmoscope. Haematological examinations were performed prior to treatment and after 4, 8 and 12 weeks. Haematological examinations of 12-week blood samples included measurement of susceptibility of erythrocytes to haemolysis in hypotonic saline. Heparinized whole blood was mixed with various concentrations of hypotonic saline. After 30 minutes, intact cells were separated by centrifugation, and haemolysis was estimated in the supernatant by measuring light transmission using a photometer. Calculations of per cent haemolysis were based on tests with physiological saline (for 0% haemolysis) and distilled water (for 100% haemolysis).

Blood biochemistry parameters were determined in samples taken before the first administration and after 4, 8 and 12 weeks of administration. Urine analysis was undertaken on urine collected from each dog prior to the start of dosing and after the termination of the study. Electrocardiographic (ECG) examinations (lead II) were undertaken prior to the first administration and after weeks 4, 8 and 12. Complete necropsies were undertaken on all dogs after the 13-week dosing period. Organs were examined grossly, weighed and prepared for microscopic evaluation.

No compound-related mortality occurred during the study. No statistically significant differences in body weight were observed between the drug and control groups. A single female in the 10 mg/kg bw per day group was reported to have had reduced feed intake for a period of 10 days; this animal also displayed muscular weakness over the same period beginning in week 8. The study report indicated that

although other instances of poor appetite were observed on occasion throughout the study, the effect was not dose related. However, data on feed consumption were not included.

Two females in the 10 mg/kg bw per day group developed transient patterns of muscle weakness involving primarily the hindlimbs. In one of the females, the symptoms appeared in the 8th week and persisted for approximately 10 days, whereas in the other, symptoms appeared in the 10th week and lasted only 1 day. During the neurological examinations at 8 weeks, two males in the 10 mg/kg bw per day group were also noted to be suffering from abnormal muscle symptoms associated with the hind legs. One of these males was observed to have an awkward gait (associated with the hindlimbs), whereas the other demonstrated bilateral tremor of the hindlimbs. After the 12th week, there were no findings in the neurological examinations that distinguished drug from control groups.

ECG tracings (lead II) revealed no significant differences between test and control groups.

No significant differences were observed in haematocrit or haemoglobin levels in blood from test and control animals. After 12 weeks, mean prothrombin time was seen to be statistically significantly increased at all doses compared with controls, and mean coagulation time was seen to be increased in the 5 and 10 mg/kg bw per day groups compared with controls. After 8 weeks, mean prothrombin time was statistically significantly increased in the 10 mg/kg bw per day group, and mean coagulation time was increased only in the 2 mg/kg bw per day group. After 4 weeks, mean prothrombin time was statistically significantly decreased in the 2 mg/kg bw per day group and statistically significantly increased in the 10 mg/kg bw per day group, whereas coagulation time was statistically significantly increased in the 2 mg/kg bw per day group only. Prior to treatment, prothrombin time was statistically significantly increased in the 10 mg/kg bw per day group. All prothrombin and coagulation times are reported to be in the normal range. Overall, the effects on prothrombin and coagulation times appear to occur randomly across the groups and are not considered compound related. No effect of lasalocid sodium on haemolysis was seen. No compound-related effects were seen on differential white blood cell count.

Blood biochemistry revealed statistically significantly increased ALAT in the 10 mg/kg bw per day group after 4 and 8 weeks, but not after 12 weeks. The elevated group mean seen after 8 weeks was heavily influenced by the enzyme levels for the female that displayed muscle weakness at this time point; AP and ASAT levels were also increased in this female at this time point, but all had returned to control levels after 12 weeks. Blood urea nitrogen levels were statistically significantly decreased in the 5 mg/kg bw per day group prior to treatment, in the 2 mg/kg bw per day group after 4 weeks, in the 5 mg/kg bw per day group after 8 weeks and in the 10 mg/kg bw per day group after 12 weeks. Based on the inconsistent nature of these findings, the effects on blood urea nitrogen are not considered test compound related. The only other statistically significant effect was decreased serum chloride levels after 4 weeks in the 10 mg/kg bw per day group, after 8 weeks in all test groups and after 12 weeks in the 5 and 10 mg/kg bw per day groups. The study authors noted that serum chloride levels in the 5 and 10 mg/kg

bw per day groups were quite consistent over the study period, but that the control levels were increased after 8 and 12 weeks, resulting in relatively lower levels in the 5 and 10 mg/kg bw per day groups.The effect seen at 2 mg/kg bw per day was seen only at the 8-week time point and is not considered test compound related.

There were no findings of note in the urine analysis.

No lesions considered to be compound related were noted at gross pathology.

At necropsy, absolute spleen weights were statistically significantly increased in the 5 and 10 mg/kg bw per day groups, whereas relative spleen weights were statistically significantly increased only in the 5 mg/kg bw per day group. Absolute and relative uterus weights were statistically significantly increased in the 2 and 5 mg/kg bw per day groups, but not in the 10 mg/kg bw per day group. This effect on the uterus is not considered test compound related, as it was not dose related. Relative thyroid weights were statistically significantly increased in the 10 mg/kg bw per day group.

A full histopathological examination was performed on dogs from all groups killed after 13 weeks. Hepatic cells showed increased vacuolation in females in the 10 mg/kg bw per day group and to a lesser degree in females at 5 mg/kg bw per day; the effect was seen in three females at 10 mg/kg bw per day and in one female at 5 and 2 mg/kg bw per day as well as in the control group. The effect was not seen in males. The pathologist reported that the vacuolation was not due to lipid, glycogen or glycosaminoglycans, that it probably represents an intracellular accumulation of water, that such changes are generally reversible and that the effect was not associated with degenerative or inflammatory changes. It is noted that the effect seen in a single female at the middle dose was less pronounced than at the high dose and that in the control and 2 mg/kg bw per day animals in which vacuolation was seen, the extent of the effect was similar. This appears to suggest the occurrence of a dose–response relationship, with the greatest effect (number of animals affected and extent of the effect) seen in the high-dose animals. The pathologist concluded that the vacuolation effect is reversible and that it is therefore not biologically significant. However, as the reversibility was not demonstrated in this study, the effect at 5 mg/kg bw per day is considered to be a significant compound-related effect.

Congestion was observed in the spleen in all test animals in all groups: moderate congestion in all animals in the control and 2 mg/kg bw per day groups, moderate congestion in four animals and marked congestion in two animals at 5 mg/kg bw per day, and moderate congestion in two animals and marked congestion in four animals at 10 mg/kg bw per day. The possibility of a compound-related effect at 5 and 10 mg/kg bw per day cannot be ruled out.

Based on decreased serum chloride levels, increased spleen weights, increased congestion in the spleen and increased hepatocyte vacuolation at 5 and 10 mg/kg bw per day, the NOAEL was 2 mg/kg bw per day (Pfitzer & Swarm, 1973).

In a study reported to have been performed to GLP standards, groups of 20 Beagle dogs (10 males and 10 females) were administered lasalocid sodium in

feed at a concentration of 10, 35 or 180 mg/kg feed (equivalent to 0, 0.25, 1 and 5 mg/kg bw per day) for 24 months. A further group of 10 males and 10 females served as controls. Animals were caged individually. No formal GLP certification was included with the study report, although a quality assurance statement listing inspection dates was provided.

Animals were observed daily for physical and behavioural signs of toxicity as well as feed consumption. Body weights were measured weekly. Physical and neurological examinations (observations of the righting, patellar, flexor, extensor, visual placing response, corneal and papillary reflexes) as well as palpation for tissue masses were performed pretest and at monthly intervals. Ophthalmic examinations were performed pretest and after 2, 3, 4, 5, 6, 9, 12, 15, 18, 21 and 24 months. ECG examinations were performed pretest and after 2, 6, 7 (high dose and controls only), 12, 18 and 24 months. Haematological, blood biochemistry and urinary examinations were performed prior to treatment and after 3, 6, 9, 12, 15, 18, 21 and 24 months (biochemical analyses at 9 months were performed twice, as a new clinical chemistry analyser was introduced at that stage, and analyses were performed using both the automated and manual systems). Two animals of each sex per group were killed after 6 and 12 months, and all remaining surviving animals after 24 months. Organs were weighed and prepared for microscopic evaluation.

All animals survived until scheduled killing. No statistically significant effects on body weight were observed, and the pattern of body weights across the dose groups did not suggest a clear drug-related effect. Feed consumption was slightly reduced in the 180 mg/kg feed group over the first 8 weeks of drug administration, but the effect did not reach statistical significance. Sporadic statistically significant differences in feed consumption were seen between test and control groups, but as these occurred with no clear pattern across the doses or dosing period, they are not considered drug related.

Intermittent paralysis of the limbs was observed for a single day during week 21 in five animals (three males and two females) in the high-dose group. The animals appeared normal within 24 hours, and paralysis did not recur. Moderate tremors were reported in one female in the low-dose group for a single day during week 54 and again at week 100. As the tremors were seen in a single low-dose animal, they are not considered drug related. No other compound-related physical signs of toxicity were seen.

Ophthalmology revealed increased retinal lesions in two animals in the 180 mg/kg feed group compared with controls. Similar lesions were noted in the 10 mg/kg feed group, but not in the 35 mg/kg feed group. The lesions were first noted at interim time periods and did not progress over the course of the study. The lack of a dose–response relationship and the lack of progression over time suggest that the effect was not drug related. The reporting ophthalmologist concluded that the effects were the result of an unidentified inflammation event occurring early on in the study.

ECG examinations revealed changes at 6 months (increased PaVF amplitude and TaVF negativity); however, as similar changes were seen in animals from all groups, including controls, and as these returned to normal levels at

subsequent time points, the effect is not considered to have been drug related. Also at 6 months, one dog in the 180 mg/kg feed group displayed ECG evidence of myocardial damage. As the effect was seen in only one animal and as it was not seen at subsequent time points, it is not considered to have been drug related.

Haematology results included the following statistically significant changes from control values: decreased white blood cell counts seen in 180 mg/kg feed males at 3, 6 and 21 months (but not at 9, 12, 15, 18 or 24 months); decreased prothrombin time in 180 mg/kg feed males at 3 and 24 months only; decreased clotting time in 35 and 180 mg/kg feed females at 9 and 21 months only; and decreased prothrombin time in 35 and 180 mg/kg feed females at 24 months only. Statistically significant changes from control values were also noted at other time points, with no dose–response relationship. Overall, based on the lack of dose and time effects, none of the changes in haematological parameters is considered to represent a drug effect.

Blood biochemistry revealed statistically significantly increased AP in males at 9, 12, 18, 21 and 24 months (180 mg/kg feed). At 15 months, levels were increased, but the increase did not reach statistical significance. In females, AP was increased at 6, 9, 18, 21 and 24 months (180 mg/kg feed). At 12 and 15 months, levels were increased, but the increase did not reach statistical significance. ASAT levels were increased in 180 mg/kg feed males at 3 and 9 months only and in females at 3 months only. Glucose levels were decreased in 180 mg/kg feed females at 6 months and increased in 35 and 180 mg/kg feed females at 15 months. Glucose levels were decreased in 180 mg/kg feed males at 9 months only. Serum sodium levels were increased in 180 mg/kg feed males pretest and decreased in 180 mg/kg feed males at 6, 12 and 18 months and in 35 and 180 mg/kg feed females at 3 months. Serum calcium levels were decreased in 180 mg/kg feed males pretest and in 35 and 180 mg/kg feed females at 3 months and increased in 35 mg/kg feed females at 15 months and in 180 mg/kg feed females at 18 months. A number of other statistically significant deviations from control values were seen scattered across the dose groups and time points. Overall, the only blood biochemistry effect that occurred consistently over the study was the effect on AP, which was seen in both sexes at 180 mg/kg feed.

There were no findings of note in the urine analysis.

No lesions considered to be compound related were noted at gross pathology.

No statistically significant organ weight effects occurred with any pattern suggestive of a drug-related effect. The following organ weight changes were not statistically significant, but did occur with increasing dose and time: in males, increased absolute and relative testicular weights were seen at 180 mg/kg feed at 24 months, and decreased absolute and relative prostate weights were seen at 35 and 180 mg/kg feed at 24 months. Absolute and relative prostate weights were also decreased at 12 and 24 months, but results from these interim time points are based on examination of only two animals. In females, increased absolute and relative spleen weights were seen at all doses at 24 months and at 12 months (but only two females were examined at the interim time point), and increased absolute and relative thyroid weights were seen at all doses at 24 months.

Histopathological examination revealed only random changes, none of which was considered to be drug related. In the absence of any histopathological correlates, the organ weight findings are not considered to represent adverse drug effects.

Based on the transient intermittent paralysis of limbs seen in the 180 mg/kg feed group and on the increased AP levels seen at this dose, 180 mg/kg feed (equivalent to 5 mg/kg bw per day) is considered to be the LOAEL for this study. The NOAEL is therefore 35 mg/kg feed (equivalent to 1 mg/kg bw per day) (Hogan & Rinehart, 1980).

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mice

A 2-year chronic toxicity study, reported to have been performed to GLP standards, was conducted in CD-1 mice. Lasalocid sodium was administered in feed at concentrations of 0 mg/kg feed (control group I and control group II), 10 mg/kg feed (low-dose animals were dosed with 20 mg/kg feed for the first 5 weeks of the study, after which the dose was adjusted down), 35 mg/kg feed (mid-dose animals were dosed with 60 mg/kg feed for the first 5 weeks, after which the dose was adjusted down) and 120 mg/kg feed (equivalent to 0, 1.5, 5.25 and 18 mg/kg bw per day, respectively, after week 5). The reason for the dose change at week 5 is not explained in the study report. Eighty mice of each sex were included in each group. Mice (5–6 weeks old at study initiation) were housed in groups of five same-sex animals. Feed and water were available ad libitum. No formal GLP certification was included with the study report, although a quality assurance statement listing inspection dates was provided.

Animals were observed daily for mortality and moribundity. Body weights, feed consumption, clinical signs and incidence/location of tissue masses were determined weekly for the first 13 weeks and biweekly thereafter. Ophthalmoscopic examinations were performed on all mice prior to initiation of the study, at week 6 and on all survivors at week 104. In addition, ophthalmoscopic examinations were performed on 10 mice of each sex per group every 3 months. Necropsies were performed on all mice found dead or killed in extremis, with gross observations recorded. After 104 weeks, all remaining mice were killed and necropsied, with gross observations recorded and tissues prepared for microscopic examination (brain, thoracic spinal cord, pituitary, thyroid, thymus, adrenals, heart, kidney, stomach, duodenum, jejunum, ileum, colon, caecum, colon, pancreas, ovaries, gall bladder, uterus, testes with epididymides, prostate, lung, spleen, liver, salivary glands, mesenteric lymph nodes, urinary bladder, nerve with muscle, bone marrow, rib junction, skin with mammary gland and any unusual lesions). Tissues from 20 males and 20 females in control groups I and II and in the 120 mg/kg feed group were examined microscopically, as were all tissues with masses or unusual lesions.

In males and females, overall survival at termination of the study was comparable in the test and control groups. In males, survival rates were comparable in all groups throughout the study. In females, reduced survival was noted in the 35 and

120 mg/kg feed groups at a number of interim time points, and survival over weeks 0–51 and 0–61 was statistically significantly reduced compared with control group II.

No effect on body weight or feed consumption considered to be associated with administration of the drug was seen.

The study report indicated that no treatment-related clinical signs were noted and that the incidence of tissue masses, nodules and wart-like lesions was comparable in control and test groups. Individual findings were not reported.

Ophthalmoscopic findings were unremarkable in all groups and were not suggestive of a compound-related effect.

Gross pathology examinations performed at terminal sacrifice and on animals that died or were killed in extremis during the study did not reveal findings suggestive of a compound-related effect.

Microscopic evaluation of tissues from animals killed at termination led to similar findings across control and test groups and was not suggestive of a drug effect. No evidence of a neoplastic effect of lasalocid was seen. Similarly, no drug effect was seen in the microscopic evaluation of tissues from males that died or were killed in extremis during the study. In females that died during the study or were killed in extremis, an increased incidence of lymphosarcoma was noted in the 10 and 120 mg/kg feed groups (3 cases seen in control group I, 5 cases in control group II, 9 cases in the 10 mg/kg feed group, 4 cases in the 35 mg/kg feed group and 10 cases in the 120 mg/kg feed group). As lymphosarcoma was not seen to occur with greater incidence in the test groups in animals killed at study termination and as a dose–response relationship was not evident in animals that died or were killed in extremis during the study, it is concluded that the increased incidence of lymphosarcoma noted in the 10 and 120 mg/kg feed groups did not represent a drug effect.

It is concluded that lasalocid sodium did not show evidence of tumorigenic potential in the mouse. The top dose of 120 mg/kg feed (equivalent to 18 mg/kg bw per day) was the NOAEL (Reno et al., 1980a).

(b) Rats

A 30-month toxicity and carcinogenicity study, reported to have been performed to GLP standards, was conducted in Fischer 344 rats. Lasalocid sodium was administered in feed at concentrations of 0 (control group I and control group II), 10, 35 and 120 mg/kg feed. Within each dose group, the dose administered varied considerably over the course of the study. For example, for males in the 120 mg/kg feed group, the mean initial dose level was calculated to be 11.3 mg/kg bw per day, whereas by 120 weeks, the dose was calculated to be 5.9 mg/kg bw per day. The average doses calculated from body weight and feed consumption were 0.5, 1.8 and 6.2 mg/kg bw per day for male rats and 0.6, 2.2 and 8.1 mg/kg bw per day for female rats, respectively. Eighty-five rats of each sex per group were included in control group I and in the lasalocid sodium groups, and 55 rats of each sex were included in control group II. Animals were caged individually except during the 3-week mating periods, when they were housed in pairs, and

during lactation, when offspring were housed with the mother. Feed and water were available ad libitum. No formal GLP certification was included with the study report, although a quality assurance statement listing inspection dates was provided. The study is reported to have continued for 30 months to meet the requirements of the United States Bureau of Foods (which required continuation until 30 months or 80% mortality).

The animals included in this study were selected at birth from litters derived from parental rats administered lasalocid sodium at the same doses from 1 week prior to breeding until weaning. When the youngest litter reached day 21, animals were randomly selected to enter into the lifetime carcinogenicity study (in which they received the same dose of lasalocid sodium as received by the parental animals during breeding, gestation and lactation).

Animals were observed daily for mortality and moribundity. Body weights, feed consumption, clinical signs and incidence/location of tissue masses were determined weekly. Neurological examinations (observations of the placement, righting, grasping and pupillary reflexes) were performed pretest and at intervals of approximately 4–5 weeks (examination of pupillary reflex was undertaken only from week 9 onwards). Ophthalmoscopic examinations were performed on all rats during week 1, on 10 rats per group at intervals of 3 months, on all rats sacrificed at 26 and 78 weeks as well as on all rats at terminal sacrifice and on any rats showing abnormal findings. Haematology (haematocrit, haemoglobin, coagulation time, total and differential leukocyte counts) and urinary examinations (appearance, pH, specific gravity, ketones, glucose, occult blood, total protein, bilirubin and microscopic examination of sediment) were performed on 10 rats of each sex per group at 3-month intervals and on all rats at 26 and 78 weeks as well as on all rats at terminal sacrifice. Clinical chemistry analyses (glucose, blood urea nitrogen, calcium, potassium, chloride, ALAT, ASAT and AP) were performed on 15 rats of each sex per group at 26, 78 and 104 weeks as well as on all rats at terminal sacrifice. Gross necropsy examinations were performed on all rats found dead or killed in extremis. Scheduled interim sacrifices were performed on 15 rats of each sex per group for all lasalocid sodium groups and for control group I at 26 and 78 weeks. All remaining rats were killed at 130 weeks. Gross pathology and histopathology examinations were undertaken at interim and terminal sacrifices. Organs were weighed (brain, heart, lung, liver, spleen, kidneys, testes with epididymis/ovaries, prostate/uterus, pituitary, thyroid, adrenals) and prepared for microscopic examination (brain, pituitary, thoracic spinal cord, eyes, salivary glands, thyroid, thymus, lungs, heart, liver, spleen, kidneys, adrenals, stomach, pancreas, duodenum, jejunum, ileum, colon, caecum, mesenteric lymph node, urinary bladder, testes with epididymis/ovaries, prostate/uterus, rib junction, bone marrow, nerve with muscle and any unusual lesions). Tissues from rats in control groups I and II and in the 120 mg/kg feed group were examined microscopically, as were tissues with masses or lesions from rats in the 10 and 35 mg/kg feed groups.

The percentage of animals surviving until week 130 was highest in the 120 mg/kg feed group, but was also increased in the 35 mg/kg feed group. The effect reached statistical significance in males in the 120 mg/kg feed group and was not considered adverse.

No effect on body weight or feed consumption considered to be associated with administration of the drug was seen, although statistically significant differences between test and control groups occurred at a number of interim time points. As these effects were transient and did not display a clear dose–response relationship, they are not considered to represent a drug effect.

The study report indicated that no treatment-related clinical signs were noted and that the incidence of tissue masses, nodules and wart-like lesions was comparable in control and test groups. Individual findings were not reported.

Haematology findings consisted of decreased haematocrit in males at 10, 35 and 120 mg/kg feed at week 130. The effect was statistically significant in the 35 and 120 mg/kg feed groups, but not in the 10 mg/kg feed group. Haematocrit was also statistically significantly reduced in all three test groups at week 26, but not at any of the seven intervening time points. Haemoglobin was also reduced in males in all test groups at week 130, but the effect reached statistical significance only in the 35 mg/kg feed group. In view of the absence of a consistent effect over time and the absence of a dose–response relationship, the effects on haematocrit and haemoglobin are not considered to be drug related. No effect considered to be drug related was seen on leukocyte counts or coagulation times.

Clinical chemistry revealed statistically significantly decreased ALAT in females in the 120 mg/kg feed group at weeks 26, 104 and 130. At week 78, ALAT was also reduced in females in the 120 mg/kg feed group, but the effect was not statistically significant. Blood urea nitrogen was statistically significantly reduced in females in all three test groups at weeks 26 and 78. No effect on blood urea nitrogen was seen in females at week 104 or 130. Blood urea nitrogen was also decreased in male rats in the 120 mg/kg feed group at weeks 26, 78 and 104, but not at week 130. Statistically significantly increased glucose levels were seen in males in the 35 and 120 mg/kg feed groups at week 26 only and in females in the 120 mg/kg feed group, also only at the 26-week time point. Chloride ion levels were statistically significantly decreased in females in the 120 mg/kg feed group at weeks 26, 78 and 104 (but not at week 130) and in the 35 mg/kg feed group at weeks 78 and 104 only. The clinical chemistry effects were not seen at all time points (and most were not seen at the final time point) and were not associated with histopathology. In light of this, they are not considered toxicologically relevant.

Urine analysis did not reveal any effects considered to be test item related.

Ophthalmoscopic examinations were unremarkable and did not reveal any effects suggestive of a drug effect.

Neurological examinations revealed an increased incidence of slow righting and grasping reflexes in females in the 120 mg/kg feed group in weeks 31–49 (e.g. the incidence of slow righting reflex at week 41 was 24/69 females compared with 2/70 females in control group I). The effect was not seen at earlier or later time points. A slightly increased incidence of these findings was also noted in the 35 and 10 mg/kg feed females at similar time points, although the number of animals affected was very low. No parallel effects were noted in males at any dose. In light of the low incidence of the findings at the low and middle doses and the absence of findings in males at any dose, the slow righting and grasping reflexes

in the 10 and 35 mg/kg feed groups are not considered drug related. No statistical analyses were performed on neurological data.

A number of organ weights were statistically significantly different from those seen in the control groups, but few differences appeared drug related. At 26 weeks, the following organ weight changes were noted: in males in the high-dose group, relative brain weight and relative testes plus epididymides weight were decreased, and absolute liver weight was increased. In females in the high-dose group, absolute and relative liver weights, absolute and relative spleen weights and absolute and relative adrenal weights were increased. Absolute and relative adrenal weights were also increased in females in the mid-dose group. At 78 weeks, the following organ weight changes were noted: in males in the high- and mid-dose groups, absolute and relative thyroid weights were decreased (the decrease in absolute thyroid weights in the mid-dose group was not statistically significant). In females, absolute and relative adrenal weights were increased in the mid- and high-dose groups. At 130 weeks, the following organ weight changes were noted: in males, absolute and relative testes plus epididymides weights were increased in the high-dose group. In females, absolute and relative liver weights were increased in the mid- and high-dose groups, absolute and relative adrenal weights were increased in the mid- and high-dose groups (although the effect was not statistically significant) and relative heart weights were increased in the high-dose group. The organ weight findings did not occur consistently over the course of the study, nor were they associated with histopathological findings. The organ weight effects are therefore not considered to be of toxicological significance.

No effects likely to be drug related were observed in the gross pathology examinations.

Microscopic examination revealed no findings suggestive of a drug effect.

In conclusion, lasalocid sodium did not demonstrate tumorigenic properties in this study. Based on a transient impairment of righting and grasping reflexes seen in females between weeks 31 and 49 at 120 mg/kg feed (equal to 8.1 mg/kg bw per day), the NOAEL was 35 mg/kg feed (equal to 2.2 mg/kg bw per day) (Reno et al., 1981).

2.2.4 Genotoxicity

Six in vitro studies were performed to examine the genotoxic potential of lasalocid sodium. The results are summarized in Table 1.

A non-GLP study was performed examining deoxyribonucleic acid (DNA) repair in *Bacillus subtilis* (rec-assay). Two strains of the bacteria were used: M45, a recombination deficient mutant (Rec–) strain derived from the wild-type H17 strain, and strain H17, with normal recombination capacity (Rec+). Agar plates were inoculated with the bacteria, covered with a paper disc containing 20 µL drug and incubated at 37 °C for 24 hours before the growth-inhibited zone was measured. Five plates were used for each dose. Lasalocid sodium (dissolved in dimethyl sulfoxide [DMSO]) was tested at concentrations of 1, 10 and 100 µg/plate, 4-nitroquinolone-*N*-oxide (the positive control, dissolved in DMSO) was tested at a concentration of 10 µg/plate and kanamycin sulfate (a negative control protein synthesis inhibitor substance, dissolved in distilled water) was tested at a concentration of 10 µg/plate.

Table 1. Results of genotoxicity assays on lasalocid sodium

Test system	Test object	Concentration	Results	Reference
DNA repair deficiency (rec-assay)	*Bacillus subtilis*	1–100 µg/plate	Negative	Yamashita et al. (1977)
Ames test[a]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537, TA1538 *Escherichia coli* B/r WP2, WP2 hcr–	100–2 000 µg/plate	Negative	Yamashita et al. (1977)
Mutagenicity[a]	*Saccharomyces cerevisiae*	0.05–5.0 mg/mL incubation mixture	Negative	Albertini & Woreth (1988)
Forward mutation assay[a]	Chinese hamster lung V79 cells (*HGPRT* locus)	1–20 µg/mL incubation mixture without metabolic activation[b] 1–60 µg/mL incubation mixture with metabolic activation	Negative	Strobel (1989a)
Unscheduled DNA synthesis	Primary hepatocytes	0.5–12.5 µg/mL[c]	Negative	Strobel (1989b)
Chromosomal aberration test[a]	Cultured human peripheral blood lymphocytes	2–8 µg/mL without metabolic activation[d] 2–10 µg/mL with metabolic activation[e]	Negative	Dresp (1989)

DNA: deoxyribonucleic acid; HGPRT: hypoxanthine–guanine phosphoribosyltransferase; S9, 9000 × *g* supernatant fraction from rat liver homogenate

[a] Both with and without rat liver S9 fraction.

[b] At concentrations greater than 15.0 µg/mL, cytotoxicity did not allow meaningful evaluation.

[c] At concentrations greater than 3.0 µg/mL, cytotoxicity did not allow meaningful evaluation.

[d] At concentrations greater than 5.0 µg/mL, cytotoxicity did not allow meaningful evaluation.

[e] At concentrations greater than 6.0 µg/mL, cytotoxicity did not allow meaningful evaluation.

4-Nitroquinolone-*N*-oxide induced greater growth inhibition in the Rec– strain than in the Rec+ strain (the difference in growth inhibition between the two strains was reported as 7.0 mm). Kanamycin induced only slightly greater growth inhibition in the Rec– strain (the difference in inhibition between the two strains was reported as 1.7 mm). The difference in growth inhibition between the two strains seen in lasalocid sodium–treated plates was less than that seen with the negative control kanamycin: at lasalocid sodium concentrations of 1, 10 and 100 µg/plate, the difference in inhibition between the two strains was 0.2, 0.5 and 0.2 mm, respectively. The results indicate that lasalocid sodium was not DNA damaging at these doses (Yamashita et al., 1977).

A non-GLP study was performed examining lasalocid sodium's ability to induce mutations in *Salmonella typhimurium* strains TA100, TA1535 (base pair substitution–sensitive strains), TA98, TA1537 and TA1538 (frameshift mutation–sensitive strains) and in *Escherichia coli* strains B/r WP2 and WP2 hcr– (base pair substitution–sensitive strains, with the latter lacking excision repair capacity), both with and without metabolic activation.

Lasalocid sodium was tested at concentrations of 100, 200, 500, 1000 and 2000 µg/plate. Each concentration was tested in triplicate.

Positive controls were diethylsulfate for TA1535 and B/r WP2 (without metabolic activation), 9-aminoacridine for TA1537 (without metabolic activation), 2,4-dinitrophenylthiocyanate for TA1538 (without metabolic activation), 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide for TA98, TA100 and WP2 hcr– (without metabolic activation), 2-aminoanthracene for TA98, TA100, TA1535, TA1537 and WP2 hcr– (with metabolic activation) and 2-acetylaminofluorene for TA1538 (with metabolic activation). Kanamycin sulfate was used as a negative control for all strains. All drugs with the exception of kanamycin sulfate were dissolved in DMSO; kanamycin sulfate was dissolved in distilled water.

The number of revertants seen following incubation of tester strains with lasalocid sodium remained at background levels in all cases, whereas positive controls induced marked increases in the number of revertants.

Lasalocid sodium did not show evidence of mutagenic activity in this assay, although slight evidence of cytotoxicity was seen at concentrations above 500 µg/plate, limiting the strength of the conclusions that can be drawn in relation to the higher doses (Yamashita et al., 1977).

Lasalocid sodium was tested for mutagenic potential in yeast (*Saccharomyces cerevisiae* D7) with and without metabolic activation in a study reported to be GLP compliant. The induction of mitotic gene conversion was monitored by the appearance of tryptophan non-requiring colonies (on selective media), reverse mutation induction was monitored by the appearance of isoleucine non-requiring colonies (on selective media) and mitotic crossing-over was assessed by visual detection of pink and red colonies that occurred due to the formation of homozygous cells expressing the genotypes ade 2-40/ade 2-40 (red) and ade 2-119/ade 2-119 (pink) from the originally heteroallelic condition ade 2-40/ade 2-119, which forms white colonies. Induction of mitotic gene conversion and reverse mutation was assessed in both the logarithmic growth phase of cells and the stationary growth phase.

Lasalocid sodium was tested at concentrations ranging from 0.05 to 5.0 mg/mL incubation mixture. Positive controls were 4-nitroquinolone-*N*-oxide (at a concentration of 0.2 µg/mL incubation mixture) for systems without metabolic activation and cyclophosphamide (at a concentration of 0.5 mg/mL incubation mixture) for systems with metabolic activation. Lasalocid sodium and the positive controls were dissolved in DMSO.

Lasalocid sodium concentrations greater than 1.67 mg/mL incubation mixture caused the appearance of white flakes in the incubation mixture. The tested concentrations of lasalocid sodium did not induce gene conversion, reverse gene mutation or mitotic crossing-over, whereas the responsiveness of the system was demonstrated by positive results obtained with the positive controls. The study report indicated that the concentrations tested were selected based on a toxicity prescreen. The results of the toxicity prescreen were not provided, but it was noted that the number of colonies that grew in complete media was similar in the control and lasalocid sodium–treated groups. The results indicate that lasalocid sodium was not mutagenic in *Saccharomyces cerevisiae* (Albertini & Woreth, 1988).

Lasalocid sodium was tested in a forward mutation assay in Chinese hamster lung V79 cells with and without metabolic activation in a study reported to be GLP compliant. A mutation to the gene for the enzyme hypoxanthine–guanine phosphoribosyltransferase (*HGPRT*) allows cells to proliferate in the presence of the purine analogues 6-thioguanine and 8-azaguanine, which, in non-mutants, are converted into toxic nucleoside-5-monophosphates and inhibit growth.

Lasalocid sodium was tested at concentrations of 1, 5, 10, 15 and 20 µg/mL incubation mixture in experiments without metabolic activation and at concentrations of 1, 10, 20, 40 and 60 µg/mL incubation mixture in experiments with metabolic activation. Ethyl methanesulfonate (at a concentration of 80 µg/mL) and 2-acetylaminofluorene (at a concentration of 80 µg/mL) were used as positive controls in experiments without and with metabolic activation, respectively. All three drugs were dissolved in DMSO, which also served as the negative control. Experiments were performed twice.

In the absence of metabolic activation, a concentration of 15 µg/mL reduced cell viability to 43–49% of that seen in the absence of lasalocid sodium and was considered the highest concentration that could be evaluated. In the presence of metabolic activation, a concentration of 60 µg/mL reduced viability to 19–27%.

The frequency of mutations conferring 6-thioguanine resistance was similar in negative control and lasalocid sodium–treated cells, but was substantially increased in positive control groups both with and without metabolic activation. The results indicate that lasalocid sodium was not mutagenic in this study in mammalian cells (Strobel, 1989a).

Lasalocid sodium was tested in an unscheduled DNA synthesis assay that measured incorporation of ^{3}H-labelled thymidine into nuclear DNA of primary (metabolically active) hepatocytes isolated from male Fü-albino rats. The study is reported to have been performed to GLP standards.

Lasalocid sodium was tested in a first experiment at concentrations of 0.5, 1.0, 2.5, 5.0, 10 and 12.5 µg/mL and in a second experiment at concentrations of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µg/mL. 2-Acetylaminofluorene (0.2 µg/mL) was used as a positive control. Both drugs were dissolved in DMSO, which was also used as a negative control.

Isolated rat hepatocytes were exposed to lasalocid sodium or 2-acetyl-aminofluorene for 18 hours. Cells were fixed and subjected to autoradiography, after which cells were evaluated microscopically, and the number of silver grains in the nuclei of cells not in the S-phase was counted.

Concentrations of 4.0 µg/mL and higher were seen to be cytotoxic and did not leave a sufficient number of morphologically normal cells to allow evaluation. The level of ^{3}H-labelled thymidine incorporation into nuclear DNA following exposure of hepatocytes to concentrations of lasalocid sodium ranging from 0.5 to 3.0 µg/mL did not differ from that seen with the negative control (DMSO). The level of ^{3}H-labelled thymidine incorporation into nuclear DNA following exposure of hepatocytes to the pro-mutagen 2-acetylaminofluorene was elevated, demonstrating the functionality of the assay. The results indicate that lasalocid sodium did not induce DNA damage resulting in DNA repair in rat hepatocytes (Strobel, 1989b).

Lasalocid sodium was tested for its potential to induce chromosomal aberrations in cultured human peripheral blood lymphocytes, with and without metabolic activation. The study is reported to have been performed to GLP standards.

Lasalocid sodium was tested in three experiments at concentrations ranging from 2 to 8 µg/mL without metabolic activation and in one experiment at concentrations ranging from 2 to 10 µg/mL with metabolic activation. Bleomycin sulfate and cyclophosphamide were used as positive controls in experiments without and with metabolic activation, respectively. Lasalocid sodium was dissolved in DMSO, bleomycin sulfate in 0.9% saline and cyclophosphamide in distilled water. DMSO served as a negative control.

Mitogen (phytohaemagglutinin-M)–stimulated cells were incubated with lasalocid sodium or control substances with or without S9 mix and subsequently subjected to metaphase arrest (using colcemid). Cells were fixed and prepared for microscopic examination.

In each of the three experiments without metabolic activation, only the lowest lasalocid sodium concentration (4, 2 and 5 µg/mL in the first, second and third experiments, respectively) produced a sufficient number of analysable cells, due to cytotoxicity. The incidence of chromosomal aberrations at the lowest doses was similar to that seen in negative controls, whereas bleomycin sulfate induced a significant increase in chromosomal aberrations, demonstrating the functionality of the system. In the experiment with metabolic activation, the top concentration of lasalocid sodium (10 µg/mL) induced excessive cytotoxicity. At concentrations up to 8 µg/mL, the incidence of chromosomal aberrations in lasalocid sodium–treated cells was similar to that seen in negative controls, whereas cyclophosphamide induced a significant increase in aberrations. The results indicate that lasalocid sodium did not induce chromosomal aberrations in human peripheral lymphocytes in vitro (Dresp, 1989).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration reproductive toxicity

In a pre-GLP study, groups of 288 Charles River CD rats (24 males and 24 females per group) were administered lasalocid sodium in the diet at an intended dose of 0 (control group 1 and control group 2), 1, 2, 3 or 10 mg/kg bw per day. Males were dosed from 21 days prior to mating through a 14-day mating period. Females were dosed from 21 days prior to mating through to weaning of pups. Weanling rats were selected for entry into a 13-week repeated-dose toxicity study (reviewed in section 2.2.2 above; Pfitzer & Roberts, 1975b). The present study (Hoar et al., 1974) detailed only the effects on the maternal F_0 generation as well as effects seen in the F_1 offspring up until weaning.

Dosing at the desired level was maintained by adjusting the concentration based on the average feed consumption and body weight of each group. Diets were adjusted for appropriate doses on a weekly basis. Average actual drug intake levels were calculated for each sex at each dose level on a weekly basis. Average weekly group intakes (overall averages over the entire study period) were, for males, 0.88–1.06 (0.96), 1.83–2.22 (1.92), 2.57–3.47 (2.91) and 9.07–11.48 (9.83) mg/kg bw per day at 1, 2, 3 and 10 mg/kg bw per day, respectively; and, for females, 0.85–1.23 (1.19), 1.74–2.48 (2.45), 2.81–3.95 (3.73) and 8.64–13.05 (12.38) mg/kg bw per day at 1, 2, 3 and 10 mg/kg bw per day, respectively.

Feed consumption and body weights were measured weekly. Females were also weighed at weekly intervals relative to day 0 of gestation. Litters were examined as soon as was practical after delivery for live and dead offspring and for external abnormalities. Pups were counted, weighed and sexed at birth and weighed again on days 4, 7, 14 and 21 of lactation. At day 4 of lactation, litters were culled to a constant maximum size of eight pups (four males and four females, where possible). At 21 days after delivery, pups were weaned, and 60 of each sex per dose were transferred to a 13-week repeated-dose toxicity study (see section 2.2.2 above; Pfitzer & Roberts, 1975b). Male F_0 animals were killed on day 4 of lactation, and female F_0 animals were killed after weaning and examined for implantation sites. Neither the male F_0 animals nor the pups were necropsied. Pups that died at birth or during lactation were examined for external abnormalities and prepared for detection of skeletal defects.

No compound-related effects on survival, body weight or fertility (i.e. number of pregnancies) were seen in male parental rats. In dams, body weights were statistically significantly reduced in the 10 mg/kg bw per day group only (compared with controls). No effects on reproductive parameters (length of gestation period, number of litters, litter size, number of implantation sites per litter, number of pups born alive per litter, sex distribution, percentage of pups alive on days 4 and 21 of weaning) considered to be drug related were seen. Average body weights of viable pups in the 10 mg/kg bw per day group were decreased at birth and up to weaning (21 days after birth) compared with those in the control group. Only pups that died at birth or during lactation were examined for visceral and skeletal abnormalities. A number of these animals did display abnormalities; however, as only small and

variable numbers of animals were examined in each group, it is not possible to comment on whether effects may have been compound related.

Owing to the limited range of parameters monitored, a NOAEL for parental toxicity cannot be established from this study. The only effect noted in the offspring was reduced body weights in the 10 mg/kg bw per day group, which may have been secondary to toxicity in the maternal animals. No effects on fertility or reproductive parameters were seen, and the NOAEL for reproductive toxicity in this study was therefore 10 mg/kg bw per day, the highest dose tested. However, this study can be considered as a preliminary study only, as it is not in line with accepted designs for multigeneration studies. In particular, males were not dosed over a full spermatogenic cycle (dosing for 70 days would be expected), and histopathological and functional observations on the male and female reproductive systems were not reported (Hoar et al., 1974).

A multigeneration reproductive toxicity study incorporating a teratology arm, reported to have been performed to GLP standards, was performed using Charles River CD rats. Lasalocid sodium was administered in feed at a concentration of 0 (control), 10, 35 or 120 mg/kg (equivalent to 0, 0.5, 1.75 and 6 mg/kg bw per day, respectively). Animals were caged individually except during the 3-week mating periods, when they were housed in pairs, and during lactation, when offspring were housed with the mother. Feed and water were available ad libitum. No formal GLP certification was included with the study report, although a quality assurance statement listing inspection dates was provided.

In the parental (F_0) generation, 70 rats (35 males and 35 females) per group were dosed. At weaning of the F_1 animals, 40 rats (20 males and 20 females) were randomly selected from each dose group to continue in the study and become the parents of the F_2 generation. This process was repeated at weaning of the F_2 animals, with a further 40 rats (20 males and 20 females) selected from each dose group to become the parents of the F_3 animals. The F_0 and F_1 generations were mated twice with the offspring from one of the matings used as a source of parental (F_1) animals for the subsequent generation. The additional mating of the F_0 generation provided females for use in teratology evaluations. Offspring from the second mating of the F_1 generation were discarded at weaning. The F_2 generation was mated 3 times with offspring from the first two matings killed at weaning. The third mating of the F_2 generation provided females for use in teratology evaluations.

Parental animals were observed daily for clinical signs of toxicity. Body weight, feed consumption, physical appearance and behaviour were recorded initially and at weeks 4 and 9 during the 9-week growth period. Animals were then housed in pairs for a 21-day mating period, after which males were returned to individual cages and females were transferred to nesting boxes for delivery of offspring and postnatal observation.

Naturally delivered offspring were examined, and the numbers of live and dead pups were recorded, along with body weights, sex and any abnormalities. On day 4, F_1 and F_2 litters were reduced to five males and five females (where possible). All other pups were killed and discarded without necropsy. At day 21,

approximately a third of the remaining pups from each litter were reported to have been killed and necropsied, with internal gross abnormalities recorded (however, necropsy data for F_1 and F_2 pups were not included in the study report). On the same day, 20 F_1 and F_2 pups of each sex per group were randomly selected to constitute the parental animals for the subsequent generation. Any remaining pups were killed and discarded without necropsy.

Parental males were killed and discarded after their last breeding cycle. Parental females were killed after weaning of their last litter (except for those in the teratology arm).

Following weaning of F_3 pups from one of the F_2 matings, 20 males and 20 females were randomly selected from each dose group except the 120 mg/kg feed group, from which only 14 males and 14 females were selected, killed and necropsied, with gross observations recorded. Tissues were prepared for microscopic examination.

Ten females from the F_0 generation and all females included in the third mating of the F_2 generation were entered into the teratology arm of the study. The day on which a vaginal plug or sperm in the vagina were observed was taken as day 0 of gestation. F_0 females in the teratology arm were observed on days 0, 6, 11 and 13 of gestation for appearance, behaviour and body weights. Parallel examinations of F_2 females in the teratology arm were performed on days 0, 6, 11, 15 and 19. Females in the F_0 generation were killed and caesarean sections performed on gestation day 13, and gestational parameters (numbers of corpora lutea, implantation sites, resorptions and live and dead fetuses) were recorded. Females in the F_2 generation were killed and caesarean sections performed on gestation day 19. In addition to the gestational parameters monitored in the F_0 generation, fetuses were examined externally, and weight, sex and crown–rump distance were measured. All fetuses from the F_0 and F_2 females were examined for external gross abnormalities. Approximately one third of fetuses from the F_2 females were examined for gross visceral abnormalities. The remaining two thirds of fetuses from the F_2 females were eviscerated and examined for skeletal abnormalities.

No treatment-related mortality was considered to have occurred in parental animals.

Body weights in F_0 parental animals were statistically significantly reduced in females in the 120 mg/kg feed group during the 9-week growth phase. A decrease (not statistically significant) in feed consumption was seen in these animals at week 9. Body weights in high-dose F_0 parental females were also reduced during gestation (measured from gestation days 0 to 13 in the teratology arm), but body weight gain during this period was not different in treated and control F_0 females. Feed consumption in high-dose F_0 females was also reduced during gestation, but statistically significantly only between days 0 and 6. In the F_1 and F_2 parental animals, body weights and feed consumption were not statistically significantly different in test and control groups during the growth phase, although reduced body weight was seen (not statistically significant) in F_2 females at 120 mg/kg feed. High-dose F_2 females in the teratology arm had reduced absolute body weights

throughout gestation (including on gestation day 0), and body weight gain during this period was also reduced, as was feed consumption.

With the exception of body weight effects, the study report indicated that no treatment-related clinical signs were observed in the parental generation (no log of clinical signs was provided in the study report).

The study report indicated that necropsy findings in parental animals did not suggest a treatment-related effect (no log of necropsy findings in parental animals other than those in the teratology arm was provided in the study report).

Pregnancy rates (number of pregnant females/number of females placed in breeding) and male fertility rates (number of males housed with females that became pregnant/number of males placed in breeding) were decreased in the high-dose group in all matings in all generations, but the effect was small except for in one of the matings in the F_2 generation, where it was statistically significant. No effect was seen on the number of females that delivered viable litters. The number of females with pups surviving to weaning was statistically significantly reduced in the high-dose group in one of the matings in the F_2 generation, but not in the other mating in the F_2 generation and not in other generations.

In the offspring, neonatal survival (survival up to day 4 of lactation) was decreased (not statistically significant) in F_1 animals in the high-dose group in one mating, but not in subsequent matings or generations. The percentage of pups surviving to weaning was statistically significantly reduced in high-dose F_3 animals from one mating of the F_2 generation, but the effect was not seen in pups from the other mating in this generation or in other generations. No effect on pup sex ratio was noted.

Pup body weight at 24 hours after birth was slightly reduced (not statistically significant) in the high-dose animals resulting from one of the F_0 matings, but not in pups from the second mating of F_0 animals or in subsequent generations. Pup body weight at 21 days after birth was slightly reduced in high-dose pups from all matings except one of the two F_0 matings, with a statistically significant effect seen in F_3 pups from one of the F_2 matings.

Clinical observations of pups did not reveal any differences between control and test groups, and neither did gross necropsy or histological examination of weanlings (gross necropsy and histological data were available for F_3 weanlings from one of the F_2 matings only).

In the teratology arm, pregnancy rates and male fertility were reduced in the F_2 generation in high-dose animals and to a lesser extent in low- and mid-dose animals. The effect was not statistically significant at any dose. No effects on pregnancy rate or male fertility rate were seen in the F_0 animals. The mean numbers of corpora lutea and implantations per pregnant dam were reduced in the high-dose group in the F_0 and F_2 generations and also in the mid-dose group in the F_2 generation. These reductions were not statistically significant. Implantation efficiency (number of implantations/number of corpora lutea) was statistically significantly reduced in high-dose F_0 dams. Implantation efficiency was also reduced in the mid-dose dams, but the effect was not statistically significant.

In F_2 dams, implantation efficiency was also reduced in the high-dose group, but not to a statistically significant degree. The incidence of resorptions, fetal deaths and fetal viability were similar in control and test groups in both F_0 and F_2 dams. Fetal body weights (measured in the F_3 generation only) were slightly reduced (not statistically significant) in the high-dose group. Fetal sex ratios and crown–rump distances were similar in all groups. The number and type of visceral abnormalities (hydronephrosis) were similar in control and treatment groups, and no skeletal abnormalities were seen in any group. The number of visceral variants (dilated renal pelvis and dilated brain lateral ventricles) was increased in the high-dose group (variants seen in 5/8 litters versus 4/15 litters in the control group). Similarly, the number of skeletal variants (delayed ossification) was increased in the high-dose group (variants in 4/9 litters versus 6/15 litters in the control group).

This is an old study, and consequently it does not meet all the requirements of current reproductive or developmental toxicity guidance (e.g. OECD Test Guideline 416 on the two-generation reproductive toxicity study or OECD Test Guideline 414 on the prenatal developmental toxicity study). In the high-dose group (120 mg/kg feed), reduced body weights were seen in parental females. The NOAEL for parental toxicity was 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day).

The mean numbers of corpora lutea and implantations per pregnant dam were reduced in both the high-dose (120 mg/kg feed) and mid-dose (35 mg/kg feed) groups, resulting in decreased implantation efficiency in these groups. The high-dose group also showed decreased pregnancy and fertility rates. Based on these effects, the NOAEL for reproductive toxicity was 10 mg/kg feed (equivalent to 0.5 mg/kg bw per day).

In the high-dose group (120 mg/kg feed), the number of pups surviving to weaning and the body weights of pups surviving to weaning were reduced. In the high-dose group of the teratogenicity arm, fetal weights were slightly reduced, and the incidence of visceral and skeletal variants was increased. The NOAEL for offspring and embryo/fetal toxicity was therefore 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day) (Reno et al., 1980b).

(b) Developmental toxicity

(i) Rats

The multigeneration study in rats included a teratogenicity arm. The study is described in the previous section (Reno et al., 1980b).

(ii) Rabbits

In a dose range–finding study reported to be GLP compliant, groups of six female New Zealand White rabbits were dosed orally by gavage over days 6–28 of gestation. Dose levels of lasalocid sodium made up in 0.5% carboxymethyl cellulose were 0 (controls), 1, 2 and 4 mg/kg bw per day. Control animals were dosed with 0.5% carboxymethyl cellulose. No formal GLP certification was included with the study report, although a quality assurance statement was provided.

Animals were examined daily for clinical signs of toxicity, excreta were checked at the start and end of each day for changes in output or quality of excreta, body weights were recorded (on days 4, 6, 9, 12, 15, 19, 22, 26 and 29) and feed consumption was recorded daily from day 4 of gestation. Surviving animals were killed on day 29 of gestation and subjected to gross necropsy of thoracic and abdominal contents. The reproductive tract was dissected out, and the number of corpora lutea graviditatis in the ovaries and the number and position of implantation sites in the uterus were recorded. Implantations were classified as live, a fetal death (death after approximately day 18 of gestation), a late embryonic death (embryonic remains are visible) or an early embryonic death (only placental remains are visible). Fetuses were examined externally for visible abnormalities, and the total weight of the live fetuses in the litter was recorded.

Analysed concentrations of the dosing formulations used in the initial days of the study were found to be up to 33% lower than the nominal concentrations in the 1 and 2 mg/kg bw per day groups but only up to 5% lower than the nominal concentration in the 4 mg/kg bw per day group. Dosing solutions were amended for the remainder of the study.

Reduced/pale faecal output was seen in 4/6, 5/6, 6/6 and 6/6 animals in the 0, 1, 2 and 4 mg/kg bw per day groups, respectively (possibly related to the dosing volume of 10 mL/kg bw), over the first days of administration and resolved before the termination of the study.

One animal in the 2 mg/kg bw per day group was killed prematurely after aborting its single fetus. This was not considered to be test substance related.

Body weight gains were reduced in the 1, 2 and 4 mg/kg bw per day groups in a dose-dependent manner (greater reduction in body weight gain at 4 mg/kg bw per day than at 2 mg/kg bw per day, and greater reduction at 2 mg/kg bw per day than at 1 mg/kg bw per day). Feed consumption was markedly reduced at 4 mg/kg bw per day and slightly reduced at 1 and 2 mg/kg bw per day. In all test groups, feed consumption was most markedly reduced on days 7 and 8.

No clear effect of lasalocid sodium on pregnancy rate or preimplantation loss was seen. At 4 mg/kg bw per day, there was a decrease in the number of mean live implantations resulting from an increased number of early and late embryonic deaths. No effect on the number of live implantations was seen at 1 or 2 mg/kg bw per day. Fetal weight was reduced at 2 and 4 mg/kg bw per day. Reduced fetal weights were also noted at 1 mg/kg bw per day, but the study authors argued that this may be a reflection of an increased litter size seen at this dose. One animal in the 4 mg/kg bw per day group produced a pup with a shortened tail with a small skin flap.

Overall, it is concluded that lasalocid sodium was associated with a dose-related decrease in body weight and feed consumption seen at all doses. At 4 mg/kg bw per day, there was a decreased number of mean live implantations (possibly secondary to maternal effects), and fetal weights were reduced at 1, 2 and 4 mg/kg bw per day (but at 1 mg/kg bw per day, these may have been a consequence of larger litter size). Based on the results of this dose range–finding

study, doses of 0, 0.5, 1 and 2 mg/kg bw per day were selected for the pivotal developmental toxicity study in rabbits (Clubb & Sutherland, 2003a).

In a developmental toxicity study reported to be GLP compliant, groups of 24 female New Zealand White rabbits were dosed orally by gavage over days 6–28 of gestation. Dose levels of lasalocid sodium made up in 0.5% carboxymethyl cellulose were 0 (controls), 0.5, 1 and 2 mg/kg bw per day. Control animals were dosed with 0.5% carboxymethyl cellulose. The dosing volume in all groups was 10 mL/kg bw. No formal GLP certification was included with the study report, although a quality assurance statement was provided.

Animals were examined daily for clinical signs of toxicity, excreta were checked at the start and end of each day for changes in output or quality of excreta, body weights were recorded (on days 4, 6, 9, 12, 15, 19, 22, 26 and 29) and feed consumption was recorded daily from day 4 of gestation. Surviving animals were killed on day 29 of gestation and subjected to gross necropsy. Premature decedents underwent necropsy at the time of death (fetuses of these animals were not weighed and were examined externally only).

In scheduled necropsies, thoracic and abdominal contents were examined macroscopically. The reproductive tract was dissected out, and the number of corpora lutea graviditatis in the ovaries and the number and position of implantation sites in the uterus were recorded. Implantations were classified as live, a fetal death (death after approximately day 18 of gestation), a late embryonic death (embryonic remains are visible) or an early embryonic death (only placental remains are visible). Fetuses were killed, and each live fetus was identified within the litter and its weight recorded. Fetuses were examined externally for visible abnormalities, including macroscopic examination of eyes and cranial bones (following removal of skin over these areas).

Each fetus was dissected and examined for abnormalities of the thoracic and abdominal viscera. The cranium was sectioned to allow inspection of the brain. The sex of each fetus was recorded. Skeletons were prepared and examined for skeletal abnormalities and for the extent of ossification.

Reduced/altered (size and colour) faecal output was seen in 3/24, 9/24, 8/24 and 20/24 animals in the control, 0.5, 1 and 2 mg/kg bw per day groups, respectively. Red staining was noted in the cages of three animals in the 2 mg/kg bw per day group, although there were no obvious effects on pregnancy outcome. One animal in the control group was found dead (cause of death unknown), and one animal in the 2 mg/kg bw per day group was killed (the animal had not eaten since 2 days before the start of the study); neither of the deaths was attributed to treatment.

Body weight gains were reduced (compared with controls) in the 0.5, 1 and 2 mg/kg bw per day groups in a dose-dependent manner. In the 2 mg/kg bw per day group, body weight gain was reduced over the entire dosing period (with mean body weight on day 29 being similar to that seen on day 6). In the 1 mg/kg bw per day group, body weight gain was reduced, particularly over the initial 6 days of

treatment, and in the 0.5 mg/kg bw per day group, body weight gain was slightly lower than that of controls, particularly during early gestation. Feed consumption was reduced in all test groups in a dose-related manner.

No clear effect of lasalocid sodium on pregnancy rate was seen. At 2 mg/kg bw per day, there was a small increase in preimplantation loss (17% versus 9% in the control group). Preimplantation loss was also slightly increased in the 1 mg/kg bw per day group (13%) and the 0.5 mg/kg bw per day group (11%), but the increases seen in these dose groups are too small to be concluded to be test substance related. The absolute number of live implantations as well as the percentage of live implantations were reduced in the 2 mg/kg bw per day group (72% versus 89% in the control group), with a parallel increase in the number of early embryonic deaths (19% versus 7% in the control group) and late embryonic deaths (4% versus 1% in the control group). In the 1 and 0.5 mg/kg bw per day groups, the percentage of live implantations was marginally different from that seen in controls (84%, 85% and 89% at 1, 0.5 and 0 mg/kg bw per day, respectively). Mean fetal weight was reduced in all test groups (43.0, 41.8, 38.0 and 33.9 g in the 0, 0.5, 1 and 2 mg/kg bw per day groups, respectively), although the reduction at 0.5 mg/kg bw per day was small and is not considered to represent a drug effect.

A greater incidence of fetuses with forelimb flexure was noted in the 1 and 2 mg/kg bw per day groups (three occurrences in two litters at 2 mg/kg bw per day, a single occurrence at 1 mg/kg bw per day and none at 0.5 mg/kg bw per day or in controls). A single occurrence of enlarged bile duct and absent spleen and gall bladder was noted at 2 mg/kg bw per day.

An increased incidence of corneal opacity was seen in fetuses in the 2 mg/kg bw per day group (four occurrences in four litters versus a single occurrence in the control group). An increased incidence of bifurcated/misshapen gall bladders was noted in all test groups (five occurrences in four litters at 2 mg/kg bw per day, two occurrences in two litters at 1 mg/kg bw per day, four occurrences in three litters at 0.5 mg/kg bw per day and none in the control group). The abnormalities in the gall bladder did not occur in a dose-related manner, and the study report indicated that they are common (although they were not seen in concurrent controls in this study) and should not be considered to represent a test-related effect. Historical control data from the laboratory were provided. These consisted of the incidence of bilobed/bifurcated/clubshaped/misshapen gall bladders in one study per year between 2000 and 2012. The incidence of the abnormality varied from zero occurrences per study to four occurrences in two litters in one study (in which 140 fetuses from 18 litters were examined, producing an incidence of 2.86% of fetuses or 11% of litters). The incidence of the abnormality in the current study was 3.47% of fetuses (17.4% of litters) at 2 mg/kg bw per day, 1.28% of fetuses (10% of litters) at 1 mg/kg bw per day and 2.45% of fetuses (13.6% of litters) at 0.5 mg/kg bw per day. The incidence rates for the 0.5 and 1 mg/kg bw per day doses are considered to be comparable with that seen in the historical control data. However, the incidence seen at 2 mg/kg bw per day exceeds that seen in historical controls, and consequently the possibility that it may have been drug

related cannot be ruled out. No other visceral abnormalities were considered to have been possibly test substance related.

A number of minor skeletal abnormalities/variants were noted at 1 and 2 mg/kg bw per day, as well as an increased incidence of incomplete ossification at 2 mg/kg bw per day.

Minor skeletal abnormalities/variants included, in fetuses in the 1 and 2 mg/kg bw per day groups, increased incidence of jugal(s) connected/fused to maxilla (10 occurrences in five litters at 2 mg/kg bw per day, 7 occurrences in four litters at 1 mg/kg bw per day, a single occurrence at 0.5 mg/kg bw per day and 4 occurrences in four litters in the control group). An increased incidence of complete 13th supernumerary rib was seen at 1 and 2 mg/kg bw per day (86 occurrences in 22 litters at 2 mg/kg bw per day, 61 occurrences in 17 litters at 1 mg/kg bw per day, 50 occurrences in 14 litters at 0.5 mg/kg bw per day and 52 occurrences in 15 litters in controls). An increased incidence of displaced pelvic girdle was also noted at 2 mg/kg bw per day (17 occurrences in 22 litters at 2 mg/kg bw per day versus 37 occurrences in 10 litters in controls). An increased incidence of unossified/incompletely ossified cranial bones, hyoid, odontoid process, pubes, digital bones, epiphyses and astragalus was seen at 2 mg/kg bw per day.

A NOAEL for maternal effects could not be established from this study, as effects on faeces, body weight gain and feed consumption were seen at all doses. This is likely to be a result of the known sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract. In relation to embryo/fetal toxicity, the LOAEL was considered to be 1 mg/kg bw per day, based on decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants at this dose. While the possibility that these effects may have been secondary to maternal toxicity is acknowledged, based on the available data, the NOAEL for embryo/fetal toxicity was established as 0.5 mg/kg bw per day (Clubb & Sutherland, 2003b).

2.2.6 Special studies

(a) Cardiovascular and respiratory end-points

Original studies specifically examining cardiovascular and respiratory end-points were not provided. While literature reports demonstrate that lasalocid has a positive ionotropic effect on in vitro muscle preparations and in anaesthetized dogs (Pressman, 1976; Bukoski, Seidel & Allen, 1979), at the doses used in the repeated-dose and chronic toxicity studies evaluated previously in this report, no evidence of cardiovascular or respiratory toxicity was seen. An ADI derived from the toxicity studies evaluated in this report will therefore be protective against potential cardiovascular and respiratory effects.

(b) Neurological/behavioural end-points

No original studies dedicated specifically to the evaluation of the neurotoxic potential of lasalocid have been provided. However, a number of literature reports relating to the neurotoxicity of ionophores, including lasalocid, are available, and a

number of the repeated-dose and chronic toxicity studies evaluated above included an analysis of neurological effects.

Literature reports indicate lasalocid to be neurotoxic in a number of species. Safran, Alzenberg & Bark (1993) reported a cluster of 10 cases of neurotoxic findings in dogs, 8 of which were suspected to have consumed dog food contaminated with lasalocid. Clinical signs are reported to have included

> progressive, bilaterally symmetrically ascending muscle weakness which progressed from the hind to the forelimbs followed by quadriplegia, hyporeflexia, and hypotonia. In 7 dogs, the respiratory muscles were affected to various degrees, causing dyspnoea and apnea in the most severe case. The mental status and cranial nerve function appeared to be normal. Pain perception was maintained. The dogs' ability to wag their tails was not affected. These neurological signs were consistent with a generalised lower motor neuron deficit.

To support their suspicions that the effects were the result of lasalocid poisoning, the authors purposely exposed two healthy dogs to lasalocid (at 10 and 15 mg/kg bw) via the diet. Starting 6–8 hours after ingestion, the dogs exhibited a gradual onset of clinical signs consistent with those seen in the dogs admitted to their clinic.

A further instance of suspected lasalocid poisoning in dogs was reported by Espino et al. (2003). Three bloodhounds demonstrated the following neurological symptoms:

> tetraparesia, hyporeflexia and hypotonia. The dogs' ability to wag their tails was not affected. Mental status and cranial nerve function appeared normal. Pain perception was maintained. The neurological signs were consistent with a generalised lower motor neuron deficit.

The dogs had eaten six dead broilers 1 day before symptoms emerged, and the presence of lasalocid in the broilers' food (150 mg/kg food) was subsequently confirmed. Clinical signs resolved between 8 and 12 days after exposure.

Similar clinical signs were reported in cats concluded to have been exposed to cat food containing the ionophore salinomycin (Van der Linde-Sipman et al., 1999). Histology revealed abnormalities in the peripheral nerves of the forelimbs and hindlimbs, with lesions localized in the axons, myelin sheath and Schwann cells (destruction of myelin sheath with formation of digestion chambers, collapsed axonal sheath filled with foamy macrophages and swollen Schwann cells). The cat food was found to have been contaminated at levels of 16–21 mg/kg. Assuming a body weight of 2 kg and consumption of 100 g food per day, this would represent a dose of 0.8–1.05 mg/kg bw, although the period over which the cats were exposed is not clear from the report.

The nature of the neurological findings reported in the repeated-dose and chronic toxicity studies evaluated previously in this report shows consistency with those presented in the literature reports referred to above. It is notable that in the repeated-dose and chronic toxicity studies, effects on the respiratory system were not noted, possibly because of exposure to lower doses. In addition, it is notable

that all symptoms of neurotoxicity noted in the repeated-dose studies were transient and resolved without removal of the drug.

The neurological symptoms reported in the literature reports and in the study reports evaluated previously in this monograph are consistent with those reported for other ionophores (Novilla et al., 1994).

The mechanism of action underlying the neurotoxic effects of lasalocid may not be limited to its ability to transport cations across membranes. Lasalocid (and other ionophores) is also reported to stimulate catecholamine release, even in the absence of Ca^{2+}. Perlman, Cossi & Role (1980) examined the ability of lasalocid, ionomycin (a divalent cation ionophore) and monensin (a monovalent cation ionophore) to release noradrenaline from phaeochromocytoma cells in vitro. All three stimulated the release of noradrenaline in the presence of Ca^{2+}, but lasalocid and monensin also stimulated release independently of Ca^{2+}. While the stimulation of noradrenaline release by monensin was greatly reduced in Na^{+}-free media, the removal of Na^{+} had a much less marked effect on lasalocid-stimulated noradrenaline release. Decreasing pH from 7.4 to 6.5 potentiated the effect of lasalocid. Lasalocid is a weak acid and in its protonated form is uncharged and so will have increased lipid solubility, which would facilitate its entry into cells. The authors concluded that catecholamine secretion by ionophores may take place by several mechanisms. Lasalocid may stimulate catecholamine release by Ca^{2+}-dependent, Na^{+}-dependent and pH-dependent mechanisms.

Consistent with the above, lasalocid has been reported to stimulate 5-hydroxytryptamine release from platelets in vitro in both the presence and absence of Ca^{2+}, by directly forming a lipophilic complex with 5-hydroxytryptamine and transporting it across the platelet membrane. This was observed at concentrations below those that stimulate Ca^{2+} uptake by cells. Lasalocid acts as a transporter of biogenic amines as well as a Ca^{2+} transporter. By stimulating Ca^{2+}-mediated exocytosis, it can stimulate the release of acetylcholine release at the neuromuscular junction (Wörner & Brossmer, 1975; Pressman, 1976).

Lasalocid has also been reported to induce morphological changes and degeneration in neuronal cells in cerebral cultures at concentrations that do not damage non-neuronal (glial) cells. Lasalocid was observed to induce Ca^{2+} influx in the neuronal cell. The effects were blocked by MK-801, an *N*-methyl-D-aspartate (NMDA) receptor antagonist, suggesting involvement of the NMDA receptor/ channel in lasalocid neurotoxicity (Safran et al., 1993).

(c) Microbiological effects

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and that complies with International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) Guideline 36 (GL36) was used by the Committee to determine the need to establish a microbiological ADI for lasalocid sodium (VICH, 2004). The decision-tree approach initially seeks to determine if there may be microbiologically active lasalocid sodium residues entering the human colon. If the answer is “no” to the questions in any of the first three steps, then no

microbiological ADI is necessary. However, should such residues be present, then two end-points of public health concern are to be considered: (1) disruption of the colonization barrier and (2) increase of the population(s) of resistant bacteria. At step 4 of the decision-tree process, it is possible to provide scientific justification to eliminate testing (i.e. the need for a microbiological ADI) for either one or both end-points. Step 5 is where a microbiological ADI would be determined. Should a microbiological ADI not be necessary, then the toxicological ADI would be used. The Committee evaluated studies on minimum inhibitory concentration (MIC) susceptibility, faecal binding interaction and biological activity of lasalocid sodium and the potential for the development of lasalocid sodium resistance and used the decision-tree to answer the following questions in the assessment of lasalocid sodium.

Step 1: Are residues of the drug and/or its metabolites microbiologically active against representatives of the human intestinal flora?

Yes, lasalocid sodium is microbiologically active against some bacterial genera and species representative of the human intestinal flora.

Lasalocid sodium is a polyether ionophore antibiotic used in veterinary medicine as a feed additive to treat poultry for the prevention of coccidiosis caused by *Eimeria* spp. Lasalocid sodium is not used in human medicine. Lasalocid sodium is highly active against Gram-positive bacteria and some obligate anaerobes. Lasalocid sodium is inactive against many Gram-negative aerobic and facultative anaerobic enteric bacteria, including Enterobacteriaceae. The antimicrobial spectrum of activity for lasalocid sodium against bacteria representative of the human intestinal microflora has been reported. The minimum concentrations of lasalocid sodium required to inhibit the growth of 50% of organisms (MIC_{50} values) have been determined on several different culture media against 84 bacterial strains, many of which are representative of the human gastrointestinal tract microflora (Table 2). The methodology employed was standard MIC agar dilution, as described by the National Committee for Clinical Laboratory Standards (NCCLS, 2004) for anaerobic bacteria. The MIC_{50}s of lasalocid sodium for a wide range of bacteria were as follows: *Bifidobacterium* spp., 0.25 µg/mL; *Eubacterium* spp., 0.125 µg/mL; *Clostridium* spp., 0.125 µg/mL; *Peptostreptococcus* spp., 0.063 µg/mL; *Lactobacillus* sp., 0.125 µg/mL; *Enterococcus* spp., 0.5 µg/mL; *Streptococcus* spp., 0.063 µg/mL; *Bacteroides* sp., 4 µg/mL; *Escherichia coli*, > 128 µg/mL; *Proteus* spp., > 128 µg/mL; and *Salmonella* spp., > 128 µg/mL. There were differences between MIC_{50} values for the different media that could be explained by binding of lasalocid sodium to proteins. The Wilkins-Chalgren medium not supplemented with blood was found to be the most appropriate for the assessment of the MIC_{50} of lasalocid sodium. Some tests were done with Wilkins-Chalgren medium supplemented with sheep's blood on different agar media. The MIC_{50} values were lower when blood was not added to the Wilkins-Chalgren medium to support growth (Table 2) (McConville, 1998).

Table 2. MIC_{50}s of lasalocid sodium against 84 strains of bacteria, many representing the normal human intestinal microbiota, by culture medium

Genus	Species	ATCC No.	MIC_{50} (µg/mL)			
			WC agar	WCB	RCA	TJA
Bifidobacterium	*adolescentis*	15703	0.5	2	–	–
		15704	0.25	1	–	–
		15705	< 0.063	0.5	–	–
		15706	0.25	0.5	–	–
	infantis	15697	0.5	1	–	–
		15702	0.125	1	–	–
		25962	< 0.063–0.25	0.2–4	–	–
	breve	15698	0.5	1	–	–
		15700	0.125	1	–	–
		15701	0.5	2	–	–
Eubacterium	*cylindroides*	25728	0.25	4	–	–
		27803	0.125	1	–	–
		27804	0.063	0.25	–	–
		28705	0.125	0.5	–	–
	lentum	43055	0.25	2	–	–
		25559	0.50	2	–	–
	dolichrum	29143	0.125	1	–	–
		29144	0.125	1	–	–
		33656	0.063–0.25	8	–	–
	rectale	35183	0.25	2	–	–
Clostridium	*difficile*	43594	0.063–0.25	2	–	–
		43596	0.063–0.125	2	–	–
		43597	0.063–0.125	2	–	–
		43598	0.063–0.125	2	–	–
	perfringens	9081	0.25	8	–	–
		12918	–	4	8	–
		12919	0.125–0.25	4	–	–
	breve	12920	0.125–0.5	8	–	–
		19574	0.125–0.25	8	–	–
		43150	0.125–0.5	2–8	NR	–

Table 2 (continued)

Genus	Species	ATCC No.	MIC_{50} (µg/mL)			
			WC agar	WCB	RCA	TJA
Peptostreptococcus	*hydrogenalis*	49630	< 0.063	0.5	–	–
	magnus	14955	0.125	1	–	–
		15794	0.125	1	–	–
		29328	< 0.063	1	–	–
		53516	< 0.063	1	–	–
	prevotii	9321	0.125	2	–	–
		14952	< 0.063	1	–	–
	productus	27340	< 0.063	0.5	–	–
		35244	< 0.063	0.5	–	–
		43917	< 0.063	0.25–0.5	–	–
Lactobacillus	*acidophilus*	4356	0.125	0.5	–	–
		4357	0.125	NR	–	–
		33200	NR	4	–	–
Enterococcus	*faecalis*	19083	0.5–1	16	–	–
		19948	0.25–0.5	–	–	–
		23241	0.5–1	–	–	–
		27274	0.5–1	–	–	–
		29212	0.5–1	–	–	–
		49532	0.5	–	–	–
		49533	0.125–0.5	–	–	–
		49757	0.25–0.5	–	–	–
	faecium	6569	0.5–1	–	–	–
Streptococcus	*agalactiae*	624	0.063–0.25	4	–	–
		NCTC 8542	0.125	–	–	–
		NCTC 9409	0.125	–	–	–
		NCTC 9412	0.063–0.125	–	–	–
	bovis	43143	0.25	4	–	–
		43144	0.125–0.25	8	–	–
		49133	0.125–0.25	4	–	–
	pyogenes	8058	< 0.063	4	–	–
		19615	< 0.063	2	–	–
		21059	< 0.063	1	–	–

Table 2 (continued)

Genus	Species	ATCC No.	MIC_{50} (µg/mL)			
			WC agar	WCB	RCA	TJA
Bacteroides	*fragilis*	29768	4	–	–	–
		43858	4	–	–	–
		43860	4	–	–	–
Fusobacterium	*prausnitzii*	27766	NR	NR	NR	–
		27768	NR	NR	> 128	–
	nucleatum	NCTC 10562	–	16	16	–
		NCTC 11326	–	NR	64	–
	ulcerans	NCTC 12111	–	128	128	–
		NCTC 12112	–	8	–	–
	varium	NCTC 10560	–	> 128	> 128	–
Escherichia	*coli*	884	> 128	–	–	–
		8739	> 128	–	–	–
		10798	> 128	–	–	–
Proteus	*mirabilis*	7002	> 128	–	–	–
		8427	> 128	–	–	–
	vulgaris	33420	128	–	–	–
Salmonella	*enteriditis*	4931	> 128	–	–	–
		13076	> 128	–	–	–
		31194	> 128	–	–	–
	typhimurium	NCTC 73	> 128	–	–	–
		NCTC 74	> 128	–	–	–
		NCTC 8298	> 128	–	–	–

ATCC: American Type Culture Collection; MIC_{50}: minimum concentration required to inhibit the growth of 50% of organisms; NCTC: National Collection of Type Cultures; NR: no result obtained; RCA: reinforced clostridial agar; TJA: tomato juice agar; WC: Wilkins-Chalgren agar; WCB: Wilkins-Chalgren agar containing 5% weight per volume defibrinated sheep's blood
Source: McConville (1998)

In a more recent GLP-compliant study (Pridmore, 2004a), the MIC of lasalocid sodium was determined against 30 bacterial strains, comprising 10 isolates from each of three groups of *Bacteroides*, *Fusobacterium* and *Peptostreptococcus* spp. genera representing the normal human intestinal microbiota. All

strains were sourced from the faecal microbiota of healthy unmedicated humans. The test system was standardized agar dilution MIC methodology using quality control strains as described in the NCCLS guidelines, now called the Clinical and Laboratory Standards Institute (CLSI) guidelines. To assess the effect of bacterial density on lasalocid sodium activity, each MIC was determined using two inoculum levels, 10^5 and 10^9 colony-forming units (cfu)/mL, for each strain. Lasalocid sodium activity against each bacterial group is summarized in Table 3. MIC_{50}, MIC_{90} and geometric mean of MIC values were calculated for each bacterial group (Table 4). In tests using the higher bacterial inoculum density, lasalocid sodium exerted little or no antibacterial activity against *Bacteroides* spp. (MIC_{50} value of 128 µg/mL). This is consistent with the known spectrum of activity for lasalocid sodium, which has low activity against Gram-negative bacteria. Lasalocid sodium activity was clearly demonstrable against the other two bacterial groups tested at the higher inoculum density. *Fusobacterium* was the most susceptible group (MIC_{50} of 1 µg/mL), whereas *Peptostreptococcus* was less susceptible, with a MIC_{50} of 4 µg/mL. At the low inoculum density, lasalocid sodium MICs against *Bacteroides* spp. (MIC_{50} of 32 µg/mL) and *Peptostreptococcus* spp. (MIC_{50} of 2 µg/mL) were lower than those obtained using the higher inoculum density. *Fusobacterium* spp. did not grow well when tested at a lower inoculum density. Thus, lasalocid sodium MIC_{50} values were not obtained for this microorganism when tested at 10^5 cfu/mL.

Table 3. MICs of lasalocid sodium against 30 strains of anaerobic bacteria representing the normal human intestinal microbiota

Strain description	DWC code[a]	High inoculum density		Low inoculum density	
		Lasalocid sodium MIC (µg/mL)	Inoculum density (cfu/mL)	Lasalocid sodium MIC (µg/mL)	Inoculum density (cfu/mL)
Bacteroides vulgatus	9795	128	9×10^8	64	9×10^4
Bacteroides merdae	9863	64	4×10^9	16	4×10^5
Bacteroides uniformis	9865	128	2×10^9	32	2×10^5
Bacteroides eggerthil	9888	64	5×10^9	32	5×10^5
Bacteroides stercoris	9891	> 128	3×10^9	64	3×10^5
Bacteroides caccae	9898	> 128	2×10^9	64	2×10^5
Bacteroides vulgatus	9900	128	5×10^8	32	4×10^4
Bacteroides ovatus	9907	> 128	2×10^9	64	2×10^5
Bacteroides distasonis	9912	64	3×10^9	16	3×10^5
Bacteroides uniformis	9923	128	2×10^9	64	2×10^5
Fusobacterium nucelatum	9861	1	1×10^7	No growth	1×10^3
Fusobacterium nucelatum	9854	1	8×10^8	No growth	8×10^4
Fusobacterium necrogenes	9885	8	6×10^8	4	6×10^4
Fusobacterium necrophorum	9890	2	1×10^9	No growth	1×10^5
Fusobacterium necrogenes	9895	1	2×10^9	No growth	2×10^5

Table 3 (continued)

Strain description	DWC code[a]	High inoculum density		Low inoculum density	
		Lasalocid sodium MIC (µg/mL)	Inoculum density (cfu/mL)	Lasalocid sodium MIC (µg/mL)	Inoculum density (cfu/mL)
Fusobacterium necrophorum	9902	1	2×10^7	No growth	2×10^3
Fusobacterium nucelatum	9903	64	7×10^8	64	7×10^4
Fusobacterium nucelatum	9908	128	8×10^9	64	8×10^5
Fusobacterium necrophorum	9914	4	2×10^9	No growth	2×10^5
Fusobacterium nucelatum	9921	1	2×10^9	No growth	2×10^5
Peptostreptococcus anaerobius	9791	4	1×10^8	2	1×10^4
Peptostreptococcus asaccharolyticus	9776	4	2×10^9	2	2×10^5
Peptostreptococcus asaccharolyticus	9783	4	3×10^9	2	3×10^5
Peptostreptococcus asaccharolyticus	9786	2	5×10^8	2	4×10^4
Peptostreptococcus asaccharolyticus	9787	4	4×10^8	2	4×10^4
Peptostreptococcus magnus	9781	4	4×10^8	2	4×10^4
Peptostreptococcus magnus	9792	2	6×10^8	2	6×10^4
Peptostreptococcus micros	9774	1	6×10^8	0.5	6×10^4
Peptostreptococcus magnus	9780	2	1×10^9	2	1×10^5
Peptostreptococcus sp.	9624	8	2×10^9	4	2×10^5

cfu: colony-forming unit; MIC: minimum inhibitory concentration
[a] Code identifying strains held within the Don Whitley Scientific Ltd culture collection.
Source: Pridmore (2004a)

To determine the microbiological ADI, both the McConville (1998) (Table 2) and the Pridmore (2004a) (Table 3) MIC data were used in the evaluation of the minimum inhibitory concentration derived from the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which the drug is active (MIC_{calc}).

The genera with a MIC_{50} – *Eubacterium* (0.125 µg/mL; McConville, 1998), *Bacteroides* (32 µg/mL; Pridmore, 2004a), *Bifidobacterium* (0.25 µg/mL; McConville, 1998), *Fusobacterium* (1 µg/mL; Pridmore, 2004a), *Peptostreptococcus* (2 µg/mL; Pridmore, 2004a), *Clostridium* (0.125 µg/mL; McConville, 1998), *Enterococcus* (0.5 µg/mL; McConville, 1998) and *Lactobacillus* (0.125 µg/mL; McConville, 1998) – were used to determine the MIC_{calc}.

Table 4. Summary of MIC parameters for lasalocid sodium against bacterial genera tested in the study by Pridmore (2004a)

Bacterial genus	MIC parameter	High inoculum density	Low inoculum density
Bacteroides	MIC range (µg/mL)	64 to > 128	16–64
	MIC_{50} (µg/mL)	128	32
	MIC_{90} (µg/mL)	> 128	64
	Geometric mean (µg/mL)	104	39
Fusobacterium	MIC range (µg/mL)	1–128	[Not determined: only three results available]
	MIC_{50} (µg/mL)	1	
	MIC_{90} (µg/mL)	64	
	Geometric mean (µg/mL)	3.7	
Peptostreptococcus	MIC range (µg/mL)	1–8	0.5–4
	MIC_{50} (µg/mL)	4	2
	MIC_{90} (µg/mL)	4	2
	Geometric mean (µg/mL)	3	2

MIC: minimum inhibitory concentration; MIC_{50}: minimum concentration required to inhibit the growth of 50% of organisms; MIC_{90}: minimum concentration required to inhibit the growth of 90% of organisms
Source: Pridmore (2004a)

Step 2: Do residues enter the human colon?

Yes. A number of residue studies using ^{14}C radiolabelling to detect total residues or analytical chemistry methods to detect parent lasalocid sodium have been conducted in chicken, turkey, quail, pheasant and eggs, as described in the residue report (FAO JECFA Monographs 15). In poultry, 90–95% of an oral dose of lasalocid sodium is excreted in faeces. Muscle contains little or no lasalocid sodium–derived residue, regardless of the period between withdrawal of medication and slaughter. However, residues may be present at low levels in offal, fat and skin. Pharmacokinetic studies in mice and rats indicated rapid absorption of lasalocid sodium, with 90–95% of the oral dose excreted in the faeces. Therefore, lasalocid sodium–related residues could enter the colon of a person ingesting tissues from treated animals.

Step 3: Do the residues entering the human colon remain microbiologically active?

Yes. Lasalocid sodium is the main compound entering the colon when the drug is ingested. However, lasalocid sodium residue should have very reduced activity within the colon of the consumer, as it will become substantially bound to faecal material.

To determine the effect of faecal binding on the antibacterial activity of lasalocid sodium, selected lasalocid concentrations of 0, 1, 2, 5, 10, 20, 50 and 100 µg/mL were incubated for up to 8 hours with increasing concentrations of sterilized human faeces (0%, 10%, 20% and 50% weight/volume [w/v] in Mueller Hinton Broth, collected from three individual donors) (Pridmore, 2004b). These human volunteers were healthy and received no antibiotic therapy during the 3 months prior to the faecal sample collection. Lasalocid sodium activity was determined using *Enterococcus faecalis* ATCC 29212 as an indicator organism, as it is susceptible to lasalocid sodium. The antibacterial activity of the supernatant obtained from centrifugation of the incubation mixture was assessed for the presence or absence of indicator organism growth before and after 24 and 48 hours of incubation. Control experiments indicated that without prior drug exposure to faecal suspensions, a lasalocid sodium concentration of 1 µg/mL consistently inhibited *E. faecalis* growth at each sampling point. Following drug exposure to 10% faeces at the various time intervals, the initial lasalocid sodium concentration required in the test system to inhibit *E. faecalis* growth after interaction with faeces increased to greater than 100 µg/mL. These data suggest that greater than 99% of the initial lasalocid sodium concentration was bound to faeces. All three faecal samples had maximal (> 99%) binding of lasalocid sodium at 10%, 20% and 50% concentrations. The degree of lasalocid sodium binding was rapid and remained constant throughout the 8-hour incubation period, indicating that the binding was irreversible. Based on this in vitro study (Table 5), it was determined that the binding of lasalocid sodium residues to 10%, 20% and 50% w/v concentration faecal material would be likely to exceed 99% in comparison with the activity in the absence of faeces. However, as no additional lasalocid sodium faecal binding interaction studies were conducted to validate and confirm the microbiological assay results (e.g. an HPLC/mass spectrometry chemical assay to directly detect lasalocid sodium remaining in the supernatant or bound to the faecal pellet), a conservative conclusion from the lack of lasalocid sodium activity under the test study protocol conditions would be that it is reduced by greater than 90% by contact with faecal material in the colon. In addition, lasalocid sodium is poorly absorbed, with 90–95% of an oral dose excreted in the faeces of experimental animals. Therefore, a value of 10% or 0.1 availability would be appropriate to use in calculating the microbiological ADI.

Table 5. Growth of* Enterococcus faecalis *ATCC 29212 in supernatants derived from lasalocid sodium/faeces mixtures at the 30-minute faecal binding period

		Lasalocid concentration (µg/mL)							
		0	1	2	5	10	20	50	100
Concentration of faeces (%)	0	+	–	–	–	–	–	–	–
	10	+	+	+	+	+	+	+	+
	25	+	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+	+

Key: + = growth after 24 hours
– = no visible growth after 48 hours
Note: The data from the other time intervals tested are identical to the results found at the 30-minute faecal binding period.
Source: Pridmore (2004b)

Step 4: Is there any scientific justification to eliminate testing for either one or both end-points of concern (i.e. disruption of the colonization barrier or resistance development)?

Lasalocid sodium does not appear to select for true acquired resistance in bacteria and is not used in human medicine. Results from the in vitro studies determining the potential for antimicrobial resistance to lasalocid sodium indicate that the development of resistance and cross-resistance to a number of commonly used antimicrobials in veterinary and human medicine is unlikely (Devriese et al., 1997; Wheadon, 2002, 2003). Thus, the only potential adverse effect on human intestinal microbiota would be disruption of the colonization barrier as the end-point of concern for determining the microbiological ADI. Interestingly, the majority of lasalocid sodium residues in the colon are bound to faeces and biologically inactive. Therefore, lasalocid sodium residues are not likely to disrupt the colonization barrier of the human intestinal microflora following the consumption of edible products from poultry. However, as there is potential for trace levels of lasalocid sodium to occur in the gastrointestinal tract, a microbiological ADI for lasalocid sodium residues was determined.

Step 5: Derivation of a microbiological ADI using the VICH GL36 approach

The formula for calculating the microbiological ADI is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated no-observed-adverse-effect concentration (NOAEC, or MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence

limit of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the genera with a MIC_{50} – *Eubacterium* (0.125 µg/mL), *Bacteroides* (32 µg/mL), *Bifidobacterium* (0.25 µg/mL), *Fusobacterium* (1 µg/mL), *Peptostreptococcus* (2 µg/mL), *Clostridium* (0.125 µg/mL), *Enterococcus* (0.5 µg/mL) and *Lactobacillus* (0.125 µg/mL) – the MIC_{calc} is 0.228 µg/mL.

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Lasalocid sodium is poorly absorbed and is excreted in faeces primarily in unchanged form. Lasalocid sodium binds rapidly and extensively to faecal contents; therefore, the value is 0.10.

Body weight: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for lasalocid sodium is calculated as indicated below:

$$\text{Upper bound of the ADI} = \frac{0.228\ \mu g/mL \times 220\ g}{0.10 \times 60\ kg\ bw}$$

$$= 8.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–8.4 µg/kg bw could be derived from in vitro MIC susceptibility testing and bioavailability studies.

2.3 Observations in humans

No observations in humans were identified.

3. COMMENTS

The Committee considered data on pharmacodynamics, pharmacokinetics, short-term and long-term toxicity, genotoxicity, reproductive and developmental toxicity, carcinogenicity and microbiological safety. In addition to the sponsor's submission, a number of studies were retrieved from the published literature. Although most of the studies submitted to the Committee pre-date GLP implementation, the overall package of data was sufficient to allow the derivation of a robust ADI. Those studies that were not performed to GLP standards are identified in this report.

3.1 Biochemical data

Following oral administration of a single radiolabelled dose of lasalocid sodium to mice, radioactivity was rapidly absorbed and excreted. Peak concentrations of radiolabelled material were seen in whole blood 15 minutes after administration, and levels had declined to background within 24 hours. The half-life of elimination of radioactivity in whole blood was 3 hours. Radioactivity was widely distributed to tissues, with the highest concentrations seen in liver, where they peaked 1 hour after administration. Approximately 95% of radioactivity was excreted in the faeces, and approximately 1% in urine, within 24 hours. A similar pattern was seen following multiple oral administrations, with radioactivity peaking in whole blood 30 minutes after the last dose and declining to background levels by 24 hours. Tissue levels were highest in the liver, where they remained detectable 48 hours after administration. Seventy-seven per cent of radioactivity was excreted in faeces within 4 hours of the last dose, and 95% within 24 hours. Excretion was observed to be more rapid in female mice than in male mice, with radioactivity in faeces peaking between 4 and 8 hours in females and between 8 and 12 hours in males.

The pattern of pharmacokinetic behaviour in rats following a single oral administration of radiolabelled lasalocid sodium was comparable to that seen in mice, with rapid absorption and excretion and a wide distribution of radioactivity in tissues. Whole blood radioactivity peaked at 3 hours, and the half-life of elimination was 4.8 hours. Radioactivity was widely distributed to tissues, with the highest levels seen in the liver, where it peaked at approximately 6 hours after administration. Approximately 85% of the administered dose was excreted in faeces within 24 hours, and approximately 1% was excreted in urine over the same period. Similar results were seen after seven daily oral doses.

In bile duct–cannulated male rats administered a single oral dose of radiolabelled lasalocid, approximately 61% of the dose was absorbed. Approximately 59% of the dose was excreted in bile within 48 hours.

In a comparative metabolism study in pig, dog, rat, mouse, chicken and turkey, the radioactive metabolite profile was similar in the faecal and liver fractions, although the relative proportions varied. The only component identified was lasalocid A, which represented the major component of the TRR in the faeces and liver in all species.

Although other residues were not identified, they were present at low levels and are considered to be minor.

3.2 Toxicological data

Critical studies are summarized in Table 6.

The acute toxicity of lasalocid sodium has been investigated in a number of species. Oral LD_{50} values were 146, 122, 33 and 40 mg/kg bw in the mouse, rat, neonatal rat and rabbit, respectively. The increased sensitivity of the rabbit may be due to the increased sensitivity of this species to effects of antimicrobial drugs on the intestinal microflora.

Table 6. Studies relevant to risk assessment

Species/study type (route)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Mouse				
Two-year study of toxicity and carcinogenicity (dietary)	0, 1.5, 5.25, 18	No relevant findings	18[a]	–
Rat				
Thirty-month toxicity and carcinogenicity study (dietary)	Males: 0, 0.5, 1.8, 6.2 Females: 0, 0.6, 2.2, 8.1	Increased incidence of impaired righting and grasping reflexes in females	2.2	8.1
Multigeneration reproductive toxicity study, including teratogenicity study (dietary)	0, 0.5, 1.75, 6	Parental toxicity: Reduced body weights	1.75	6
		Reproductive toxicity: Decreased numbers of corpora lutea and implantations, decreased implantation efficiency	**0.5[b]**	1.75
		Offspring toxicity: Decreased number of pups surviving to weaning, decreased body weight of pups surviving to weaning	1.75	6
		Embryo and fetal toxicity: Decreased fetal weights, increased incidence of visceral and skeletal variants	1.75	6
Rabbit				
Developmental toxicity study (gavage)	0, 0.5, 1, 2	Maternal toxicity: Decreased body weight gain, decreased feed consumption and altered faecal output	–	0.5[c,d]
		Embryo and fetal toxicity: Decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants	**0.5[b]**	1

Table 6 (continued)

Species/study type (route)	Doses (mg/kg bw per day)	Critical end-point	NOAEL (mg/kg bw per day)	LOAEL (mg/kg bw per day)
Dog				
Two-year toxicity study (dietary)	0, 0.25, 1, 5	Transient intermittent paralysis of limbs and increased serum AP	1	5

AP: alkaline phosphatase; bw: body weight; LOAEL: lowest-observed-adverse-effect level; NOAEL: no-observed-adverse-effect level

[a] Highest dose tested.

[b] Pivotal study value (Reno et al., 1980b; Clubb & Sutherland, 2003b).

[c] Lowest dose tested.

[d] Maternal toxicity was likely due to the sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract. It is not considered appropriate to consider the maternal toxicity in relation to derivation of an ADI.

Lasalocid sodium was not irritating to the skin of rabbits but caused corneal irritation, conjunctival redness and chemosis when applied to the eyes.

Lasalocid sodium did not cause skin sensitization in the guinea-pig maximization test.

In a non-GLP 13-week study in rats, lasalocid sodium was administered in the diet at concentrations adjusted to achieve doses of 0, 2, 5 and 20 mg/kg bw per day. Based on reduced feed consumption, increased liver to body weight ratios and increased haemosiderin in the liver in females, the LOAEL was 5 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a non-GLP 13-week study in weanling rats, lasalocid sodium was administered in the diet at concentrations adjusted to achieve doses of 0, 1, 2, 3 and 10 mg/kg bw per day. Based on increased AP levels seen in males at all doses at week 13, the LOAEL was 1 mg/kg bw per day. No NOAEL could be established. It is noted, however, that the low-dose effect on AP seen in this study was not seen in other rat studies.

In a non-GLP 13-week study performed in weanling rats that had been exposed to lasalocid sodium in utero (parents were exposed prior to and during mating, gestation and lactation), the substance was administered in the diet at concentrations adjusted to achieve doses of 0, 1, 2, 3 and 10 mg/kg bw per day. Based on increased haemosiderin seen in the liver of males and (predominantly) females, the LOAEL was 3 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a non-GLP 13-week toxicity study in dogs, lasalocid sodium was administered in gelatine capsules at doses of 0, 2, 5 and 10 mg/kg bw per day. Transient muscle weakness involving primarily the hindlimbs was noted in animals at the top dose only. Based on decreased serum chloride levels, increased spleen weights, increased congestion in the spleen and increased hepatocyte vacuolation, the LOAEL was 5 mg/kg bw per day, and the NOAEL was 2 mg/kg bw per day.

In a 2-year toxicity study in dogs, lasalocid sodium was administered in the diet at concentrations of 0, 10, 35 and 180 mg/kg feed (equivalent to 0, 0.25, 1 and 5 mg/kg bw per day, respectively). Based on the transient intermittent paralysis of limbs occurring on a single day and on increased AP levels, the LOAEL was 180 mg/kg feed (equivalent to 5 mg/kg bw per day), and the NOAEL was 35 mg/kg feed (equivalent to 1 mg/kg bw per day).

In a 24-month carcinogenicity study, mice were administered lasalocid sodium in feed at a concentration of 0, 10 (low-dose animals were dosed with 20 mg/kg feed for the first 5 weeks of the study, after which the dose was adjusted downward), 35 (mid-dose animals were dosed with 60 mg/kg feed for the first 5 weeks, after which the dose was adjusted downward) or 120 mg/kg feed (equivalent to 0, 1.5, 5.25 and 18 mg/kg bw per day, respectively, after week 5). Lasalocid sodium did not show evidence of tumorigenic potential. The NOAEL was 120 mg/kg feed (equivalent to 18 mg/kg bw per day), the highest dose tested.

In a 30-month toxicity and carcinogenicity study, rats were administered lasalocid sodium in feed at a concentration of 0, 10, 35 or 120 mg/kg (equal to mean doses of 0, 0.5, 1.8 and 6.2 mg/kg bw per day for males and 0, 0.6, 2.2 and 8.1 mg/kg bw per day for females, respectively). The animals used in this study were weanlings bred from parental animals administered the same doses of lasalocid sodium during breeding, gestation and lactation. Lasalocid sodium did not demonstrate tumorigenic properties in this study. Based on a transient impairment of righting and grasping reflexes seen in females between weeks 31 and 49, the LOAEL was 120 mg/kg feed (equal to 8.1 mg/kg bw per day), and the NOAEL was 35 mg/kg feed (equal to 2.2 mg/kg bw per day).

Lasalocid sodium did not show evidence of genotoxic potential in a range of in vitro tests covering the end-points of gene mutation and chromosomal aberration. Although there was no in vivo test for chromosomal effects, the Committee considered that this was unnecessary in view of the existing genotoxicity and carcinogenicity data.

In a multigeneration reproductive toxicity study incorporating a teratology arm, rats were administered lasalocid sodium in feed at a concentration of 0, 10, 35 or 120 mg/kg (equivalent to 0, 0.5, 1.75 and 6 mg/kg bw per day, respectively). At weaning, F_1 animals were randomly selected to become the parents of the F_2 generation; at weaning of F_2 animals, these were randomly selected to become parents of the F_3 generation. F_0 animals and F_2 animals were mated more than once in order to allow for evaluations of teratology. In the high-dose group (120 mg/kg feed), reduced body weights were seen in parental females. The NOAEL for parental toxicity was 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day). The mean numbers of corpora lutea and implantations per pregnant dam were reduced in both the high-dose (120 mg/kg feed) and mid-dose (35 mg/kg feed) groups, resulting in decreased implantation efficiency in these groups. The high-dose group also showed decreased pregnancy and fertility rates. Based on these effects, the NOAEL for reproductive toxicity was 10 mg/kg feed (equivalent to 0.5 mg/kg bw per day). In the high-dose group (120 mg/kg feed), the number of pups surviving to weaning and the body weights of pups surviving to weaning were reduced. In the high-dose group of the teratogenicity arm, fetal weights were slightly reduced, and the incidence of visceral and skeletal

variants was increased. The NOAEL for offspring and embryo/fetal toxicity was therefore 35 mg/kg feed (equivalent to 1.75 mg/kg bw per day).

In a developmental toxicity study in rabbits, lasalocid sodium was administered by oral gavage over days 6–28 of gestation at a dose of 0, 0.5, 1 or 2 mg/kg bw per day. A NOAEL for maternal effects could not be established, as soft stools and effects on body weight gain and feed consumption were seen at all doses. This is likely the result of the known sensitivity of rabbits to antibacterial effects on the microflora of the gastrointestinal tract, and consequently it is not considered appropriate to consider the maternal toxicity in relation to the derivation of an ADI. The LOAEL for embryo and fetal toxicity was 1 mg/kg bw per day, based on decreased litter weights, increased incidence of forelimb flexure and minor skeletal abnormalities/variants at this dose. Although the Committee acknowledges the possibility that these effects may have been secondary to maternal toxicity, it considers the NOAEL for embryo and fetal toxicity to be 0.5 mg/kg bw per day.

No original studies dedicated specifically to the evaluation of the neurotoxic potential of lasalocid sodium were provided. Literature data indicate that polyether ionophores, including lasalocid, do have neurotoxic potential. In line with this, a number of the repeated-dose studies summarized above did include examination of neurological end-points. Evidence of neurotoxicity, consisting of transient patterns of muscle weakness involving primarily the hindlimbs, was seen in the 13-week and 2-year dog studies. These effects were seen only at the highest dose and resolved spontaneously, despite continued administration of the drug. In addition, in the 30-month rat study, impairment of the righting and grasping reflexes was seen. A clear effect was evident only at the top dose and, as with the effects seen in the dog, resolved spontaneously, despite continued administration of the drug.

No observations in humans were identified.

3.3 Microbiological data

A JECFA decision-tree approach that was adopted by the sixty-sixth meeting of the Committee (Annex 1, reference *181*) and complies with VICH GL36 (VICH, 2004) was used by the Committee to determine the need for, and to establish, if necessary, a microbiological ADI for lasalocid sodium. Studies of microbiological activity against bacterial strains representative of the human colonic flora were evaluated.

The microbiological ADI was derived from in vitro MIC data as described in VICH GL36. The strains needed to determine the MIC_{calc}, which is the minimum inhibitory concentration derived from the lower 90% confidence limit for the mean MIC_{50} of the relevant genera for which the drug is active, were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. The genera with a MIC_{50}, including *Eubacterium* (0.125 µg/mL), *Bacteroides* (32 µg/mL), *Bifidobacterium* (0.25 µg/mL), *Fusobacterium* (1 µg/mL), *Peptostreptococcus* (2 µg/mL), *Clostridium* (0.125 µg/mL), *Enterococcus* (0.5 µg/mL) and *Lactobacillus* (0.125 µg/mL), were used to determine the MIC_{calc}.

Lasalocid sodium residues may be present at low levels in meat products consumed by humans; therefore, lasalocid sodium–related residues could enter the colon of a person ingesting edible tissues from treated animals. The Committee used pharmacokinetic studies and faecal binding studies to determine the fraction of the oral dose available to the human intestinal microflora. Lasalocid sodium was poorly absorbed after oral administration in animals and also binds extensively (> 90%) to faecal contents. Therefore, low levels of lasalocid sodium residues entering the human colon will remain biologically active. There is potential for disruption of the colonization barrier in the human gastrointestinal tract, as MIC values for some of the most relevant and predominant genera in the gastrointestinal tract were susceptible to lasalocid sodium. Lasalocid sodium does not appear to select for resistance in bacteria, and carboxylic polyether ionophores are not used in human medicine.

The formula for calculating the microbiological ADI is as follows:

$$\text{Upper bound of the ADI (µg/kg bw)} = \frac{MIC_{calc} \times \text{Mass of colon content}}{\text{Fraction of oral dose available to microorganisms} \times \text{Body weight}}$$

The equation terms are derived as described below.

MIC_{calc}: In accordance with Appendix C of VICH GL36, calculation of the estimated NOAEC (MIC_{calc}) for colonization barrier disruption uses MIC values from the lower 90% confidence limit of the mean MIC_{50} for the most relevant and sensitive human colonic bacterial genera. The strains needed to determine the MIC_{calc} were chosen according to these guidelines, which state that an intrinsically resistant bacterial genus should not be included. Based on the MIC_{50} values for *Eubacterium* (0.125 µg/mL), *Bacteroides* (32 µg/mL), *Bifidobacterium* (0.25 µg/mL), *Fusobacterium* (1 µg/mL), *Peptostreptococcus* (2 µg/mL), *Clostridium* (0.125 µg/mL), *Enterococcus* (0.5 µg/mL) and *Lactobacillus* (0.125 µg/mL), the MIC_{calc} is 0.228 µg/mL.

Mass of colon content: A value of 220 g is based on the colon content measured from humans.

Fraction of oral dose available to microorganisms: It is recommended that the fraction of an oral dose available for colonic microorganisms be based on in vivo measurements for the drug administered orally. Alternatively, if sufficient data are available, the fraction of the dose available for colonic microorganisms can be calculated as 1 minus the fraction (of an oral dose) excreted in urine. Human data are encouraged, but, in their absence, non-ruminant animal data are recommended. In the absence of data to the contrary, it should be assumed that metabolites have antimicrobial activity equal to that of the parent compound. The fraction may be lowered if the applicant provides quantitative in vitro or in vivo data to show that the drug is inactivated during transit through the intestine. Lasalocid sodium is poorly absorbed and is excreted in faeces of experimental animals, primarily in unchanged form. Lasalocid sodium binds rapidly and extensively (> 90%) to faecal contents; therefore, the fraction of oral dose available to microorganisms would be 0.10.

Body weight: The body weight of an adult human is assumed to be 60 kg.

The upper bound of the microbiological ADI for lasalocid sodium is therefore calculated as follows:

$$\text{Upper bound of the ADI} = \frac{0.228\ \mu g/mL \times 220\ g}{0.10 \times 60\ kg\ bw} = 8.4\ \mu g/kg\ bw$$

Therefore, a microbiological ADI of 0–8.4 µg/kg bw could be derived from in vitro MIC susceptibility testing and bioavailability studies.

4. EVALUATION

The Committee considered the toxicological effects of lasalocid sodium to be the most relevant for the purpose of establishing an ADI. A toxicological ADI of 0–5 µg/kg bw was established based on the NOAEL of 0.5 mg/kg bw per day from the developmental toxicity study in rabbits and the multigeneration reproductive toxicity study in rats, with application of an uncertainty factor of 100 for interspecies and intraspecies variability.

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