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Safety and efficacy of Avatec® 150G (lasalocid A sodium) as a feed additive for chickens for fattening and chickens reared for laying

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Abstract

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying. In 2017, the FEEDAP Panel was not able to conclude on a safe dose for chickens for fattening and chickens reared for laying and no conclusion could be drawn on the anticoccidial efficacy of the additive at the lowest proposed used level (75 mg lasalocid A sodium/kg feed) in these species. In the present assessment, the applicant submitted new tolerance and efficacy studies in chickens for fattening to address the concerns identified by the FEEDAP Panel in its former opinion. In addition, the applicant proposed to decrease the maximum of the dose range from 125 to 100 mg lasalocid A sodium/kg complete feed. Based on the additional information, the FEEDAP Panel concludes that no safe level of lasalocid A sodium from Avatec® 150G in feed for chickens for fattening can be identified. The FEEDAP Panel is not in the position to conclude on the coccidiostatic efficacy of Avatec® 150G for chickens for fattening at the lowest proposed dose level of 75 mg lasalocid A sodium/kg complete feed due to the insufficient number of studies with positive results. The conclusions are extended to chickens reared for laying.

Keywords: Coccidiostat, Avatec, lasalocid A sodium, safety, efficacy, chickens for fattening, chickens reared for laying

Requestor: European Commission

Question number: EFSA-Q-2019-00494

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

Regulation (EC) No 1831/2003[1] establishes the rules governing the Community authorisation of additives for use in animal nutrition and, in particular Article 9 defines the terms of the authorisation by the Commission.

The applicant, Zoetis Belgium SA, is seeking a Community authorisation of lasalocid A sodium as a feed additive to be used as a coccidiostat and histomonostats for chickens for fattening, chickens reared for laying, turkeys for fattening and minor avian species (pheasants, guinea fowl, quails & partridges), except laying birds. (Table 1)

Table 1: Description of the substances

Category of additive	Coccidiostat and histomonostats
Functional group of additive	Coccidiostat and histomonostats
Description	lasalocid A sodium
Target animal category	Chickens for fattening, chickens reared for laying, turkeys for fattening and minor avian species (pheasants, guinea fowl, quails & partridges), except laying birds
Applicant	Zoetis Belgium SA
Type of request	New opinion

On 16 May 2017, the Panel on Additives and Products or Substances used in Animal Feed of the European Food Safety Authority ("Authority"), in its opinion on the safety and efficacy of the product, could not conclude on the safety and on the anticoccidial efficacy of lasalocid A sodium in chickens for fattening/reared for laying, after the discussion with the Member States, it was suggested to check for the possibility to demonstrate safety.

The Commission gave the possibility to the applicant to submit complementary information in order to complete the assessment and to allow a revision of Authority's opinion. The new data has been received on 03 July 2019.

In view of the above, the Commission asks the Authority to deliver a new opinion on lasalocid A sodium as a feed additive for chickens for fattening, chickens reared for laying, turkeys for fattening and minor avian species (pheasants, guinea fowl, quails & partridges), except laying birds based on the additional data submitted by the applicant.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of additional information[2] to a previous application of the same product.[3]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of Avatec® 150G (lasalocid A sodium) is in line with the principles laid down in Regulation (EC) No 429/2008[4] and the relevant guidance documents: Guidance on the assessment of the safety of feed additives for the target species (EFSA FEEDAP Panel, 2017a) and Guidance on the assessment of the efficacy of feed additives (EFSA FEEDAP Panel, 2018).

[1] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[2] FEED dossier reference: FAD-2019-0046.
[3] FEED dossier reference: FAD-2013-0040.
[4] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and The presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

3. Assessment

The additive Avatec® 150G is a preparation of the polyether ionophore lasalocid A sodium produced by fermentation. The additive is intended for the control of coccidiosis at a dose range of 75–125 mg lasalocid A sodium/kg complete feed for chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds with a withdrawal period of 3 days.

In 2017, the FEEDAP Panel issued an opinion on the safety and efficacy of the coccidiostat Avatec® 150G (lasalocid A sodium) for chickens for fattening and chickens reared for laying. In the same opinion, the Panel also assessed the modification of the withdrawal period for all the species in which the additive is currently authorised, i.e. chickens for fattening, chickens reared for laying, turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds (EFSA FEEDAP Panel, 2017b). In this opinion, the FEEDAP Panel was not able to conclude on a safe dose for chickens for fattening and chickens reared for laying. No conclusion could be drawn on the anticoccidial efficacy of the additive at the lowest proposed used level (75 mg lasalocid A sodium/kg feed) in chickens for fattening and chickens reared for laying.

In the current assessment, the applicant submitted new tolerance and efficacy studies in chickens for fattening to address the concerns identified by the FEEDAP Panel in its former opinion (EFSA FEEDAP Panel, 2017b). In addition, the applicant proposed to decrease the maximum of the dose range from 125 to 100 mg lasalocid A sodium/kg complete feed. This opinion does not address turkeys for fattening, minor avian species (pheasants, guinea fowl, quails and partridges) except laying birds. The modification of the terms of authorisation was assessed and concluded in the former opinion (EFSA FEEDAP Panel, 2017b).

3.1. Safety

3.1.1. Safety for the target species

The applicant resubmitted the tolerance study with chickens for fattening, already assessed by the FEEDAP Panel in 2017 (EFSA FEEDAP Panel, 2017b), in which levels of lasalocid A sodium from 125 mg/kg (1.25×) to 312.5 mg/kg (3.1×) complete feed were studied.[5] Based on the results of this study (study 1), the FEEDAP Panel was not in a position to conclude on the safety of Avatec® 150G for chickens for fattening since 125 mg lasalocid sodium/kg feed resulted in a significant growth depression. In the current submission, the applicant added to the study report liver histopathology results not provided previously. The full description of the study including the new information is included in Appendix A. Liver tissue from 250 mg/kg and 312.5 mg/kg groups was excluded from the evaluation. The FEEDAP Panel notes that there was a dose-dependent increase in incidence and severity of panlobular hepatocellular hypertrophy in the two groups examined (125 and 187.5 mg/kg). No such findings were observed in the control group. These findings would not modify the conclusions drawn previously by the FEEDAP Panel.

The applicant also provided two additional tolerance studies with Avatec® 150G in chickens for fattening. Both studies were designed to establish a margin of safety of the newly proposed maximum level of 100 mg lasalocid A sodium/kg complete feed.

3.1.1.1. Study 2

A total of 360 1-day-old Ross 708 chickens (males) were randomly allocated to four treatment groups (60 birds per treatment, 6 replicates with 10 birds each + 5 spare birds) which were fed diets containing 0, 90 (0.9x maximum proposed level), 100 (1.0×) and 125 (1.25×) mg lasalocid A sodium/kg feed (analytically confirmed, see Table 2), respectively, for 35 days.[6]

The FEEDAP Panel notes that a relatively high percentage (one-third) of the birds placed initially in a pen were removed on day 7. To achieve this, small or unthrifty birds were chosen and culled; if less than the required number of birds could be chosen, birds were randomly removed. This procedure could have an impact on the body weight at day 7, but this impact cannot be assessed in the absence of data on body weight.

The basal diet consisted mainly of maize and soybean meal supplemented with methionine; the starter formulation was calculated to contain 21.1% crude protein (CP, analysed 20.2%), 0.70%

[5] Technical dossier/Annex III.1.
[6] Technical dossier/Annex III.2.

methionine (met) and 13.8 MJ metabolisable energy (ME)/kg; the grower formulation 19.9% CP (analysed 22.3%), 0.63% met and 14.1 MJ ME/kg. The starter was fed as crumbles for the first 21 days, the grower as pellets until the end of the study. The birds had ad libitum access to feed and water.

Bird health, mortality and litter conditions were recorded daily. Feed intake was measured throughout the study. Birds were weighed individually on day 0 and by pen on day 21 and day 35. Zootechnical parameters (weight gain and average daily gain, daily feed intake per bird and feed to gain ratio,) were calculated for each phase. Blood samples were taken for haematology[7] and clinical biochemistry[8] from two animals per pen on day 35. Two other animals/pen were necropsied, organ and tissue samples[9] were collected and preserved for histology. Organs were weighed and examined for lesions and abnormalities. All birds, which died in the course of the study, were necropsied.

The pen was the experimental unit for statistical purposes. Statistical analyses were conducted at the 0.05 level of significance using two-sided test. Body weight and average daily gain were analysed using a general linear mixed model for repeated measures with the fixed effects of treatment, time point and treatment by time point interaction. Average daily feed intake and feed to gain ratio, haematological, biochemical variables were analysed using a general linear mixed model with the fixed effect of treatment. Absolute and relative organ weights were analysed using a general linear mixed model with the fixed effects of treatment.

The birds reached a 35-day body weight which was in average 90% of the commercially expected value (2,029 vs. 2,255 g, based on Ross performance objectives (2019)[10]). This seems to be due to a reduced feed intake (93% of performance objectives). No relevant differences were seen in feed to gain ratio. Since the relevant zootechnical figures of the control group are below of the performance objectives for Ross 708 chicken, these parameters are less sensitive to adverse factors; however, a further depression of these control figures by the treatment with the test item, even small, would gain more weight.

Feeding lasalocid A sodium to chickens for fattening for 35 days resulted, whatever the level applied, in a significant lower final body weight, average daily gain and average daily feed intake compared to the control (Table 2). The depression measured in the 0.9× group in relation to the control group amounted to about 7% for body weight and average daily gain. The corresponding values at the use level group were about 7.5% and 9%, respectively. It is noteworthy that these differences were not seen (with exception of the 1.25× overdose group) in the starter phase. Any modified feed palatability is therefore considered highly unlikely as causative factor for the observed growth depression.

Table 2: Effect of Avatec® 150G (lasalocid A sodium) on the performance parameters of chickens for fattening and on other relevant endpoints.

	Control	**0.9×**	**1.0×**	**1.25×**
Lasalocid A sodium (mg/kg feed)				
Intended	0	90	100	125
Analysed, starter	nd	82	102	129
Analysed, grower	nd	86	97	120
Mortality(1) (n)	1	3	5	0
Performance parameters				
Final body weight (g)	2,029	1,895*	1,876*	1,569*
Average daily gain (g/bird)	57	53*	52*	44*
Average daily feed intake (g/bird)	76	72*	71*	64*
Adjusted average feed to gain ratio	1.43	1.46	1.44	1.55*

[7] Red blood count (RBC), haematocrit, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDW), thrombocyte check, white blood cell count (WBC) and differentials including heterophils, eosinophils, basophils, monocytes and lymphocytes.

[8] Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (gGT), alkaline phosphatase (AP), creatine kinase (CK), total protein, total cholesterol, blood urea nitrogen (BUN), glucose, calcium, phosphorus, magnesium, sodium, potassium, chloride, uric acid, albumin and globulin.

[9] Liver, heart, spleen, kidneys, bursa of Fabricius, crop, gizzard, small intestine, caecum, colon, sciatic nerve, pancreas, skeletal muscle, brain, lung, proventriculus, adrenals, thymus, thyroid and testis.

[10] http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-708-BroilerPO2019-EN.pdf

	Control	0.9×	1.0×	1.25×
Haematology				
Haemoglobin (g/dL)	12.2	11.1*	11.3	10.6*
Mean corpuscular haemoglobin (pg)	43.6	40.3*	41.3	39.5*
Mean corpuscular volume (fL)	119.2	113.3*	114.1	110.0*
Clinical biochemistry				
Alkaline phosphatase (U/L)	7,793	7,137	5,770	4,809*
Total protein (g/dL)	2.7	2.3*	2.4	2.2*
Albumin (g/dL)	0.8	0.5	0.6	0.6
Albumin/globulin ratio	0.6	0.5*	0.5*	0.4*
Potassium (mmol/L)	5.3	4.9*	4.8*	4.6*
Pathology (Organ weight)				
Bursa of Fabricius (relative to body weight)	0.181	0.164	0.158	0.173
Liver (relative to body weight)	2.13	2.43	2.51*	2.63*

nd: not detected.
*: Values significantly different from the control ($p \leq 0.05$).
(1): n out of 90 for the first week (including the spare birds) thereafter out of 60 birds per treatment group. Mortality in the first week was 1 in the control group, 1 in the 0.9× group and 4 in the 1.0× group.

Considering the haematology endpoints, no statistically significant differences were noted for absolute basophils, absolute eosinophils, heterophils, absolute lymphocytes, absolute monocytes, red blood cell count, red blood cell distribution width, absolute segmented heterophils and white blood cell count. Lasalocid-related changes were observed in haematocrit, Hb, MCH and MCV (Table 2). The decrease in Hb, MCH and MCV in comparison to the control group was significant for the low (0.9×) and the overdose (1.25×) levels that of haematocrit for the overdose group only (29.5 vs. 33.33%). The differences in haematology parameters were small, but appear dose related.

In biochemical variables, no statistically significant differences between the groups were found for ALT, AST, LDH, CK, gGT, albumin, globulin, glucose, total cholesterol, uric acid, Ca, P, Na, Cl and Mg. Treatment-related changes were observed in albumin to globulin (A:G) ratio and potassium serum concentrations which were significantly lower in all lasalocid-treated groups than in the control group (Table 2). Lower values of total protein were observed in the lasalocid-treated groups when compared to the control group and this decrease was statistically significant for the 0.9× and 1.25× dose level groups. The lower protein was associated with a decrease in albumin and of the A:G ratio. The differences were small and not considered of clinical relevance. Plasma alkaline phosphatase (AP) was lower in all three treatment groups when compared to the control group, although only the overdose attained statistical significance (Table 2). Alkaline phosphatase activity in birds primarily resulting from osteoblastic activity is found in higher concentrations in growing animals. Decreases in alkaline phosphatase were not considered biologically relevant.

No treatment-related findings could be identified by gross pathology. Lower relative weight for bursa of Fabricius was observed in all treatment groups compared to control group (Table 2). Although these changes were not statistically significant, the lower weights of bursa of Fabricius correlated microscopically with minimal to mild decreased lymphocytes in the low (0.9×) and the high (1.25×) dose groups. A dose-related increase in relative liver weight was observed in all treatment groups; it was statistically significant for the higher dose groups (1.0 and 1.25×) (Table 2). This increase in relative liver weight correlated microscopically to minimal to mild hepatocellular hypertrophy. There were no statistically significant differences in the relative weights of heart and kidney.

The organ weight findings and microscopic findings related to the liver and bursa of Fabricius were not considered adverse due to lack of correlative clinical pathology findings and lack of morphologic evidence of degeneration and/or necrosis.

Considering the potential of lasalocid to cause neurotoxic effects (EFSA, 2004; EMA-CVMP 2015; EFSA FEEDAP Panel, 2017b), the applicant was requested to submit histopathological examination of the brain samples preserved in the study; however, such analysis was not provided.[11]

[11] Technical dossier/Supplementary information June 2020.

3.1.1.2. Study 3

The design of study 3[12] was similar to the previous study with the following exceptions: (i) male Ross 308 chickens for fattening were used, (ii) starter feed contained 7.5% Avistart 2,[13] (iii) haematological and clinical biochemistry endpoints were not measured and (iv) feeding lasalocid for 35 days was followed by a recovery period of 7 days. The starter feed was calculated to contain 22.0% CP (analysed 24.9%), 0.89% met and 13.8 MJ ME/kg, the grower diet 19.9% CP (analysed: 22.6%), 0.63% met and 14.1 MJ ME/kg.

Birds were weighed individually on days 0, 7, 14, 21, 35, 38 and 42; feed intake was measured and the other zootechnical endpoints calculated for the corresponding intervals. On day 35, one randomly selected bird per pen was necropsied and liver tissues collected. Statistical procedure corresponds to study 2.

The birds reached a 35-day body weight which was in average 105% of the commercially expected value (2,504 vs. 2,376 g, based on Ross performance objectives (2019)).[14] Also feed intake and feed to gain ratio met the level of the performance objectives.

The results are summarised in Table 3. Twenty-five of 26 birds that were found dead or euthanised prior to study completion were necropsied and evaluated grossly. Mortality was not treatment-related. All mortalities were considered to be those commonly observed in chickens of this breed and age and were considered incidental (cardiomyopathy 2, inflammation 4, congenital leg anomaly 2, septicaemia 5, undetermined 12 of a total of 25 birds examined grossly).

The observed zootechnical parameters did not show a significant difference in the final body weight, average daily gain, feed intake and feed conversion compared to the control birds for the overall study period. The final body weights of the 0.9×, 1.0× and 1.25× dose level groups were 2, 4 and 6% lower, respectively, than in the control group.

Table 3: Effect of Avatec® 150G (lasalocid A sodium) on the performance parameters of chickens for fattening and on other relevant endpoints

	Control	0.9×	1.0×	1.25×
Lasalocid A sodium (mg/kg feed)				
Intended	0	90	100	125
Analysed, starter	nd	88	94	118
Analysed, grower	nd	93	100	120
Mortality(1) (n)	5	7	9	5
Performance parameters				
Final body weight (g)	2,504	2,458	2,416	2,361
Average daily gain (g/bird)	65	63	61	62
Average daily feed intake (g/bird)	92	92	86	88
Adjusted average feed to gain ratio	1.42	1.46	1.43	1.44
Pathology				
Liver weight (g)	59.7	62.9	53.4	52.9
Liver weight (relative to body weight)	2.42	2.60	2.40	2.87
Liver hypertrophy, hepatocellular, panlobular (n)	0	0	1	2
Liver hypertrophy/hyperplasia, Kupffer cell (n)	0	0	1	3

nd: not detected.
(1): n out of 90 for the first week (including the spare birds) thereafter out of 60 birds per treatment group. Mortality in the first week was 1 in the control group, in the 0.9× and the 1.0× group and 3 in the 1.25× group.

Overall, there were no statistically significant effects of treatment with lasalocid on absolute or relative liver weight. Although not statistically significant, there was a slight increase in relative liver weight in the 1.25× overdose group. A similar increase in liver relative to body weight was not observed after recovery (day 42).

[12] Technical dossier/Annex III.3.
[13] A highly digestible protein source, manufactured by co-processing soy and yeast, low in anti-nutritional factors, with a standardised ileal digestibility.
[14] http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross308-308FF-BroilerPO2019-EN.pdf

Study 3 cannot be considered as a complete tolerance study due to the absence of data for haematology and clinical biochemistry; however, the zootechnical and organ pathology results can be considered when deriving a final conclusion.

3.1.1.3. Synopsis of the safety studies in chickens for fattening

The zootechnical data of study 2 confirm the earlier assessment of the FEEDAP Panel (study 1) that lasalocid A sodium at concentrations of 125 mg/kg feed and above is not safe for chickens for fattening. The results of study 2 showed adverse effects on the zootechnical parameters at the proposed maximum level of 100 mg lasalocid A sodium/kg feed and below (90 mg/kg); lasalocid from 90 mg/kg feed and above significantly reduced final bodyweight, average daily gain and daily feed intake in study 2. Similar results were identified in study 3, but only numerical differences were found (not significant). The differences observed between study 2 and 3 could be due to an interaction between the diet and lasalocid treatment. Since the diet used in study 2 corresponds to a commercial type and does not show nutrient deficiencies or imbalances, the FEEDAP Panel concludes that the negative effects of Avatec® 150G on the zootechnical performance could occur under practical conditions and consequently are considered adverse.

Evidence for lasalocid-related increase of relative liver weight was seen. Histopathology identified minimal to mild hepatocellular hypertrophy. Due to lack of correlative clinical pathology, lack of morphologic evidence of degeneration and/or necrosis, and the likely reversibility of the findings (study 3) the liver findings are not considered adverse. The applicant was requested to submit histopathological examination of the brain samples preserved in the study; however, such analysis was not provided.

3.1.1.4. Conclusions on the safety for chickens for fattening

No safe level of lasalocid A sodium from Avatec® 150G in feed for chickens for fattening could be identified. This conclusion is extended to chickens reared for laying.

3.2. Efficacy

Following the opinion of the FEEDAP Panel in which no conclusion could be made on the anticoccidial efficacy of Avatec® 150G (lasalocid A sodium), the applicant provided three new floor pen studies and three new anticoccidial sensitivity tests (AST). Each study included a treatment group that received the additive at the lowest inclusion level of the proposed dose range (75 mg lasalocid A sodium/kg complete feed).

3.2.1. Floor pen studies

The three trials submitted followed a similar design (Table 4).[15] In each trial, 1-day-old chickens (Ross 308; male and female) were penned and distributed into the experimental groups. The experimental groups were: an uninfected untreated control group (UUC), an infected untreated control group (IUC), two infected Avatec®-treated groups (IT). The IT groups received feed containing 75 mg (IT75) or 85 mg lasalocid A sodium/kg feed (IT85). The intended dietary concentrations were analytically confirmed (see Table 4). The experimental diets, based on wheat, corn and soybean mill, were fed for 35 days. In the infected groups, all birds were inoculated orally via a syringe with recent field isolates of pathogenic *Eimeria* species.[16] Animal health and mortality were monitored daily. Feed intake and body weight of the animals were measured; feed to gain ratio was calculated. Samples of excreta were analysed for oocyst excretion. Selected birds (five birds per pen) were necropsied for gut lesion scoring on days 21, 28 and 35 following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The data were subjected to analysis of variance (ANOVA) using a general linear mixed model. The pen was the experimental unit for statistical purposes. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests. If the treatment effect was significant, pair-wise comparisons were made (e.g. least significant difference).

[15] Technical dossier/Section IV/Annex IV.04-06. and Supplementary information April 2020.

[16] The inocula used in floor pen trials were tested for its virulence in dose-titration studies. The doses selected (see Table 4) for trial 1 resulted in a mean lesion score of 2.0 at day 6 and a weight gain reduction of 39%, no mortality was observed; for trial 2 resulted in mean lesion scores ranging from 1.6 to 2.6 at day 7 and a weight gain reduction of 29%, no mortality was observed; for trial 3 resulted in mean lesion scores ranging from 2.7 to 3.6 at day 6, a weight gain reduction of 29% and a mortality of 20%.

Table 4: Experimental design of floor pen studies with chickens for fattening fed Avatec®

Trial no (year of conduct)	Replicates per treatment (birds per replicate)(1)	Inoculum characteristics				Feed analysis lasalocid A sodium (mg/kg feed)(2)
		Date and country of isolation	Intended dose (number of oocysts) and strain per bird		Day and mode of inoculation	
1 (2018)	12 (18)	01/2018, Denmark	558,000	*E. acervulina*	Day 15 individual inoculation	77.2/75.1/74.2 87.1/89.1/88.0
2 (2018)	10 (30)	01/2018, Denmark	57,000 43,000	*E. acervulina* *E. tenella*	Day 14 individual inoculation	75.3/77.2 91.4/87.3
3 (2018)	10 (30)	05/2018, Spain	30,000 17,000 10,000	*E. acervulina* *E. maxima* *E. tenella*	Day 14 individual inoculation	70.5/67.5 81.6/73.8

(1): In trial 1, birds were not sexed after hatching and the distribution of female and male birds occurred as hatched. In trial 2 and 3, birds were allocated to each treatment group with five pens per sex.
(2): In trial 1, birds received starter diet from day 0 to 13, grower diet from day 13 to 28 and finisher diet from day 28 to 35. In trials 2 and 3, birds received starter diet from day 0 to 13 and grower diet from day 13 until study completion.

Mortality is reported in Table 5. In trial 1, mortality was very low and not coccidiosis related. In trial 2, mortality was mainly related to coccidiosis, but no significant difference was found between IUC and the Avatec® 150G treated birds. In trial 3, only two birds died as a result of the coccidiosis infection at 6 days post-infection.

Table 5: Coccidiosis-related mortality (total mortality) in floor pen trials (n)

Trial no	Number of birds per treatment	UUC	IUC	IT75	IT85
1	216	0 (6)	0 (1)	0 (7)	0 (3)
2	300	0 (12)	34 (50)	36 (47)	37 (59)
3	300	0 (19)	2 (24)	0 (17)	0 (32)

Inoculation with *Eimeria* oocysts resulted in all trials in a significant increase of the lesion scores 7 days after infection (see Table 6). An increase of the lesion scores in the UUC group at 14 and 21 days after inoculation indicated a spread of coccidiosis in the poultry house. In trials 1 and 2, the birds treated with Avatec® 150G at 75 or 85 mg lasalocid A sodium/kg feed did not have a significantly lower mean lesion score compared to IUC birds. In trial 3, significantly lower *E. maxima* mean lesion scores were seen in the IT groups compared to IUC at day 21 (0.48 and 0.76, respectively, vs. 1.19), but no differences were seen in *E. acervulina* and *E. tenella* lesion scores.

Table 6: Lesion scores for different *Eimeria* species at different study days in floor pen trials

	***E. acervulina* lesion scores**			***E. tenella* lesion scores**			***E. maxima* lesion scores**		
	Day 21	**Day 28**	**Day 35**	**Day 21**	**Day 28**	**Day 35**	**Day 21**	**Day 28**	**Day 35**
Trial 1									
UUC	0.85^{b}	1.95^{a}	2.08^{a}	–	–	–	–	–	–
IUC	2.05^{a}	1.76^{ab}	0.93^{b}	–	–	–	–	–	–
IT75	2.52^{a}	1.31^{b}	0.80^{b}	–	–	–	–	–	–
IT85	2.15^{a}	1.30^{b}	0.83^{b}	–	–	–	–	–	–
Trial 2									
UUC	0.07^{b}	1.62^{a}	0.11	0.03^{b}	0.00^{b}	0.10	–	–	–
IUC	2.42^{a}	0.22^{b}	0.04	2.34^{a}	0.38^{a}	0.16	–	–	–
IT75	2.54^{a}	0.23^{b}	0.00	2.40^{a}	0.46^{a}	0.06	–	–	–
IT85	2.50^{a}	0.34^{b}	0.04	2.38^{a}	0.54^{a}	0.04	–	–	–

	E. acervulina lesion scores			*E. tenella* lesion scores			*E. maxima* lesion scores		
	Day 21	Day 28	Day 35	Day 21	Day 28	Day 35	Day 21	Day 28	Day 35
Trial 3									
UUC	0.46^{c}	1.60	0.60^{a}	0.12^{b}	0.32	0.12^{b}	0.56^{b}	0.80^{b}	0.42
IUC	2.27^{a}	1.06	0.10^{c}	0.90^{a}	0.24	0.20^{ab}	1.19^{a}	1.26^{a}	0.34
IT75	2.36^{a}	1.82	0.25^{bc}	0.92^{a}	0.35	0.18^{ab}	0.48^{b}	1.22^{a}	0.39
IT85	1.78^{b}	1.55	0.38^{ab}	0.90^{a}	0.35	0.33^{a}	0.76^{b}	1.06^{ab}	0.32

a,b,c: Means with different superscript letter in a column in trial are significantly different ($p < 0.05$).

In all trials, the highest oocysts excretion occurred at day 20–22 and these numbers decreased over time (see Table 7). No significant differences were observed between IUC and Avatec® 150G treated animals at any of the time points measured. A cross contamination was generally observed after day 27–29 resulting in a significantly higher oocysts excretion in the UUC birds when compared to the treated groups.

Table 7: Oocyst excretion (OPG) at different study days in floor pen trials(1)

	Day 21	Day 28	Day 35
Trial 1			
UUC	14^{b}	$40,275^{a}$	$42,199^{a}$
IUC	$3,559,568^{a}$	660^{b}	42^{b}
IT75	$1,260,277^{a}$	$1,862^{b}$	106^{b}
IT85	$2,353,026^{a}$	$1,716^{b}$	527^{b}
Trial 2			
UUC	1^{b}	$77,303^{a}$	$3,720^{a}$
IUC	$217,466^{a}$	$17,222^{b}$	684^{ab}
IT75	$199,052^{a}$	$16,677^{bc}$	314^{ab}
IT85	$236,742^{a}$	$38,517^{abc}$	132^{b}
Trial 3(2)			
UUC	$2,374^{b}$/$21,604^{c}$	19,758	184
IUC	$133,109^{a}$/$80,423^{abc}$	12,670	8
IT75	$219,429^{a}$/$141,463^{a}$	12,138	189
IT85	$201,457^{a}$/$84,344^{ab}$	16,538	14

OPG: oocyst excretion per gram excreta.
a,b,c: Means with different superscript letter in a column in trial are significantly different ($p < 0.05$).
(1): In trials 2 and 3, day 21 and day 28 refer to a period of day 20–22 and day 27–29, respectively, in which the samples were pooled.
(2): Female/male.

Zootechnical parameters are reported in Table 8. The significant differences in feed intake, daily weight gain and feed to gain ratio between UUC and IUC in all three trials (except feed intake in trial 3) confirm the challenge by *Eimeria* inoculation. Avatec® 150G treated infected birds showed improved performance parameters compared to IUC birds (daily weight gain and feed to gain ratio).

Table 8: Zootechnical parameters of chickens for fattening fed Avatec® 150G in floor pen studies

	Feed Intake (g/d)	Weight Gain (g/d)	Feed to gain ratio
Trial 1			
UUC	79^{a}	62^{a}	1.40^{a}
IUC	73^{c}	57^{c}	1.45^{b}
IT75	75^{bc}	60^{b}	1.42^{a}
IT85	76^{b}	61^{b}	1.41^{a}

	Feed Intake (g/d)	Weight Gain (g/d)	Feed to gain ratio
Trial 2			
UUC	89[a]	67[a]	1.43[a]
IUC	83[b]	64[b]	1.50[b]
IT75	84[b]	67[a]	1.45[a]
IT85	84[b]	67[a]	1.46[a]
Trial 3			
UUC	90	65[a]	1.52[a]
IUC	86	59[b]	1.56[b]
IT75	90	66[a]	1.50[a]
IT85	88	65[a]	1.51[a]

a,b,c: means with different superscript letter in a column in trial are significantly different ($p < 0.05$).

3.2.2. Anticoccidial sensitivity tests

The three tests submitted followed the same design (see Table 9).[17] Recent field isolates of *Eimeria*[18] species were used for inoculation. The birds were randomly allocated to the groups (UUC, IUC, IT). In AST-1 and AST-2, the IT group received feed containing 75 mg lasalocid A sodium/kg feed while in AST-3, two concentrations were tested; IT75 and IT85 groups received 75 and 85 mg lasalocid A sodium/kg feed, respectively. The intended dietary lasalocid A sodium concentration was analytically confirmed (Table 9). Animal health and mortality were monitored. Feed intake and body weight of the animals were measured, daily weight gain and feed to gain ratio were calculated. Final body weights were not reported. Samples of excreta were analysed for oocyst excretion. Intestinal lesions were scored following the method of Johnson and Reid (1970) (0 = no lesion, 1 = very mild, 2 = mild, 3 = moderate and 4 = severe).

The data were subject to analysis of variance (ANOVA). The pen was the experimental unit for statistical purposes. All hypothesis tests were conducted at the 0.05 level of significance using two-sided tests.

Table 9: Experimental design of ASTs with chickens for fattening fed Avatec 150G®

Trial no (year of conduct)	Replicates per treatment (birds(1) per replicate)	Inoculum characteristics				Anticoccidial treatment(2) (days of life)	Feed analysis lasalocid A sodium (mg/kg feed)
		Date and country of isolation	Intended dose (number of oocysts) per bird and strain		Day of inoculation		
1 (2017)	10 (12)	8/2017 Belgium	101,000	*E. acervulina*	12	11–19	78.4
			20,000	*E. tenella*			
			67,000	*E. maxima*			
2 (2017)	10 (12)	6/2017 UK	294,000	*E. acervulina*	14	12–20	78.4
			54,000	*E. tenella*			
			2,000	*E. mitis*			
3 (2017)	10 (12)	5/2018 Spain	34,500	*E. acervulina*	14	12–20	76.4/85.7
			25,000	*E. tenella*			
			5,500	*E. maxima*			

(1): Male and female Ross 308.
(2): Birds in the IT group were fed a basal diet supplemented with Avatec 150G. Animals in the control groups UUC and IUC received the same basal diet without inclusion of the coccidiostat.

[17] Technical dossier/Section IV/Annex IV.01-03. and Supplementary information April 2020.
[18] The inocula used in ASTs were tested for its virulence in dose titration studies. The doses selected (see Table 9) for AST-1 resulted in mean lesion scores of 1.4–2.2 at day 7 post-inoculation (PI) and a weight gain reduction of 47%, no mortality was observed; for AST-2 resulted in mean lesion scores of 0–1.8 at day 6 PI; no mortality and weight gain reduction was observed; for AST-3 resulted in mean lesion scores of 2.2–3.0 at day 6 PI and a weight gain reduction of 37%, mortality was not observed.

Mortality after challenge was low in all studies (five in AST-1 and AST-2, nine in AST 3 from a total of 720 birds in each study considering all the treatment groups); coccidiosis-related mortality was not observed.

The results of the ASTs are summarised in Table 10. Intestinal lesion scores (ILS) due to *E. maxima* improved significantly in the treated groups compared to the IUC groups in AST-1 and AST-3. ILS due to *E. acervulina* improved significantly in AST-2. Oocyst excretion also showed a significant reduction for at least one *Eimeria* species in AST-1 (*E. maxima*) and AST-2 (*E. tenella*) but not in AST-3. In all studies, performance parameters were improved in the treated groups compared to the IUC groups.

Table 10: Summary of anticoccidial sensitivity tests performed with Avatec® 150G

AST	Tr. group	Mean lesion scores(1)			OPG(2)				Daily feed intake (g)	Weight gain (g)	Feed to gain ratio
		acer	*max*	*ten*	*acer*	*max*	*ten*	*mit*			
1	UUC	0.3^{b}	0.1^{a}	NR	387^{b}	3^{b}	NR	NR	87^{b}	68^{b}	1.29^{b}
	IUC	1.5^{c}	0.8^{b}	NR	$429,519^{a}$	$49,828^{c}$	NR	NR	74^{c}	46^{c}	1.62^{c}
	IT	1.8^{a}	0.3^{c}	NR	$565,394^{a}$	992^{a}	NR	NR	79^{a}	58^{a}	1.39^{a}
2	UUC	0.4^{b}	0.3	NR	111^{b}	NR	0^{b}	3^{b}	97^{b}	68^{b}	1.45^{b}
	IUC	1.4^{c}	0.1	NR	$158,395^{a}$	NR	$4,807^{c}$	975^{a}	90^{c}	66^{c}	1.37^{a}
	IT	0.9^{a}	0.2	NR	$96,543^{a}$	NR	316^{a}	833^{a}	94^{a}	70^{a}	1.34^{a}
3(3)	UUC	0.4^{b}	0.5^{c}	0.1^{b}	$20,556^{b}/38^{b}$	NR	16^{b}	NR	88^{a}	67^{a}	1.33^{bc}
	IUC	1.8^{a}	1.7^{a}	0.5^{a}	$151,147^{a}/437,314^{a}$	NR	115^{ab}	NR	82^{b}	57^{b}	1.44^{a}
	IT75	1.8^{a}	1.0^{b}	0.6^{a}	$196,095^{a}/182,659^{a}$	NR	183^{ab}	NR	87^{a}	65^{a}	1.34^{b}
	IT85	1.7^{a}	0.8^{b}	0.6^{a}	$338,451^{a}/253,776^{a}$	NR	937^{a}	NR	83^{b}	62^{a}	1.34^{b}

acer: E. acervulina; max: E. maxima; ten: E. tenella; mit: E. mitis; NR: not reported.
a,b,c: mean in columns within a study with different superscript are significantly different ($p \leq 0.05$).
(1): Lesions were scored on day 7 post-inoculation (PI) in AST-1, and on day 6 PI in AST-2 and AST-3.
(2): oocyst per gram excreta detected at the end of the tests.
(3): For *E. acervulina* oocyst excretion female/male reported.

3.2.3. Synopsis of efficacy studies

In three floor pen studies, no significant effects of the additive were seen on the primary endpoints, i.e. mortality, lesion score and oocyst excretion with one exception; in the floor pen 3, intestinal lesions caused by *E. maxima* (but not by *E. acervulina and E. tenella*) were significantly reduced by both lasalocid levels. Average daily gain and feed to gain ratio were significantly improved in all trials; however, these secondary endpoints have only a supportive value. In summary, only one of the newly submitted studies indicates the coccidiostatic potential of lasalocid at a dietary concentration of 75 mg/kg.

In all three ASTs, coccidiosis-related mortality was not observed. Consequently, the challenge by oocyst inoculation is considered low. Lower lesion scores were reported in all three ASTs, but only related to one *Eimeria* species in each study. *E. maxima* oocyst excretion was reduced by the treatment in AST-1. In AST-2, *E. tenella* oocyst were excreted in small quantity, still significantly lower in IT than in IUC. Overall, it is concluded that the evidence, despite considered weak, confirms a coccidiostatic potential of the additive at the tested doses.

3.2.4. Conclusions on efficacy

One floor pen study and three anticoccidial sensitivity tests indicated the coccidiostatic potential of the additive. In the absence of two additional floor pen studies showing positive effects, the FEEDAP Panel is not in the position to conclude on the coccidiostatic efficacy of Avatec® 150G for chickens for fattening at the lowest proposed dose level of 75 mg lasalocid A sodium/kg complete feed. This conclusion is extended to chickens reared for laying.

4. Conclusions

No safe level of lasalocid A sodium from Avatec® 150G in feed for chickens for fattening could be identified. This conclusion is extended to chickens reared for laying.

The FEEDAP Panel is not in the position to conclude on the coccidiostatic efficacy of Avatec® 150G for chickens for fattening at the lowest proposed dose level of 75 mg lasalocid A sodium/kg complete feed due to the insufficient number of studies with positive results. This conclusion is extended to chickens reared for laying.

5. Documentation as provided to EFSA/Chronology

Date	Event
01/07/2019	Dossier received by EFSA. Additional information on Avatec® 150G (lasalocid A sodium) for chickens for fattening, chickens reared for laying. Submitted by Zoetis Belgium SA
24/07/2019	Reception mandate from the European Commission
20/08/2019	Application validated by EFSA – Start of the scientific assessment
29/01/2020	Request of supplementary information to the applicant in line Article 7(3) of Commission Regulation (EC) No 1304/2003 – Scientific assessment suspended. *Issues: efficacy*
01/04/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
25/05/2020	Request of supplementary information to the applicant in line with Article 8(1)(2) of Regulation (EC) No 1831/2003 – Scientific assessment suspended. *Issues: safety for the target species*
15/06/2020	Reception of supplementary information from the applicant - Scientific assessment re-started
01/07/2020	Opinion adopted by the FEEDAP Panel. End of the Scientific assessment

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Abbreviations

ANOVA	Analysis of variance
AST	Anticoccidial sensitivity tests
EMA	European Medicines Agency
FEEDAP	Additives and Products or Substances used in Animal Feed
OPG	Oocyst excretion

Appendix A – Tolerance study in chickens for fattening (study 1)[5]

The current text follows the version of the 2017 opinion (EFSA FEEDAP Panel, 2017b) with textual changes only for the dietary lasalocid levels taking into account the recent maximum proposed lasalocid level (100 mg lasalocid A sodium/kg feed) and considering the newly submitted data (liver histopathology).

A total of 600 1-day-old Ross 708 chickens (300 males and 300 females) were randomly allocated to five treatment groups (120 birds per treatment, 6 replicates for each gender with 10 birds each) which were fed diets containing 0, 125 (1.25× maximum proposed level), 187.5 (1.9×), 250 (2.5×) and 312.5 (3.1×) mg lasalocid A sodium/kg feed (analytically confirmed, see Table A.1), respectively, for 35 days.[19] The basal diet consisted mainly of maize and methionine supplemented soybean meal; the starter formulation was calculated to contain 21.7% crude protein (CP), 0.59% methionine (met) and 12.9 MJ metabolisable energy (ME)/kg; the grower formulation 20.0% CP, 0.55% met and 13.1 MJ ME/kg. The starter was fed as crumbles for 21 days, the grower as pellets until the end of the study. The birds had ad libitum access to feed and water. Bird health, litter conditions and mortality were recorded daily. Birds were weighed individually on day 0 and by pen on day 21 and day 35. Zootechnical parameters (feed intake, average daily feed intake, feed to gain ratio, weight gain and average daily gain) were calculated. Blood samples were taken for haematology[20] and clinical biochemistry[21] from one animal per pen on day 35. The same animals were necropsied; organ and tissue samples[22] collected and preserved for histology. Organs were weighed and examined for lesions and abnormalities. All birds, which died in the course of the study, were necropsied.

Data were analysed by a general linear mixed model with the fixed effects of treatment, sex and interaction treatment per sex. The pen was considered the statistical unit; differences were considered significant at a level of $p < 0.1$ (two-sided).

The results are summarised in Table A.1. Mortality was low (see Table A.1) for the control group and the groups fed lasalocid at 1.25×, 1.9× and 2.5× overdose and not dose related. Only the high lasalocid dose group (3.1×) showed a higher mortality rate (about 24%). Tissues of 30 birds (out of 49 dead birds) from the highest dose group were microscopically examined. Sixteen had changes consistent with lasalocid toxicity only (degeneration and regeneration of skeletal muscle, myocardial degeneration and regeneration (diagnosed as cardiomyopathy) and neuropathy) and six further birds showed changes of lasalocid toxicity plus evidence of bacterial septicaemia. The report did not allow an assignment of these 22 birds to the experimental groups.

In the overall study period (Table A.1), all treated birds performed significantly worse than the control birds. The differences in average daily gain between the lasalocid use level and the control group were small (- 4%) however significant. For the groups with the two intermediate (1.9× and 2.5×) and the high lasalocid (3.1×) levels a considerable reduction in average daily gain (−28%, −49% and −65%, respectively) and feed intake (average reduction for females and males: −19%, −38% and −54%, respectively) was seen. This resulted in a significant impairment of feed to gain ratio (by +12%, +24% and +44%, respectively) for the three groups compared to the control. No significant differences between the 1.25× overdose group and the control group were seen for feed intake of the females and feed to gain ratio for both genders. Feed intake of male birds decreased significantly in the 1.25× overdose group.

[19] Technical dossier/Supplementary information April 2015/Annexes_1_Safety_for_the_target_species.

[20] RBC (red blood count), haematocrit, haemoglobin, MCH (mean corpuscular haemoglobin), MCV (mean corpuscular volume), MCHC (mean corpuscular haemoglobin concentration), RDW (red cell distribution width), thrombocyte check, WBC (white blood cell count) and differentials, heterophils, eosinophils, basophils, monocytes and lymphocytes.

[21] AST (aspartate aminotransferase), ALT (alanine aminotransferase), LDH (lactate dehydrogenase), GGT (gamma-glutamyl transpeptidase), AP (alkaline phosphatase), CK (creatine kinase), total protein, total cholesterol, BUN (blood urea nitrogen), glucose, calcium, phosphorus, magnesium, sodium, potassium, chloride, uric acid.

[22] The following samples were collected, weighed and placed into 10% buffered neutral formalin: liver, heart, spleen, kidneys and Bursa of Fabricius. The following samples were collected and placed into formalin without being weighed: crop, gizzard, small intestine caecum and skeletal muscle.

Table A.1: Least square means of the most relevant parameters from the tolerance study 1 in chickens for fattening with lasalocid A sodium (35 days duration, six replicates per treatment and gender, one bird per replicates for serum values)

		Control	1.25×	1.9×	2.5×	3.1×
Lasalocid A sodium (mg/kg feed)						
Intended		0	125	187.5	250	312.5
Analysed, starter		nd	116	176	248	297
Analysed, grower		nd	120	186	251	304
Mortality(1)	M + F	3	7	4	6	29
Performance parameters						
Final body weight (g)	M + F	1831^{a}	1745^{b}	1349^{c}	942^{d}	678^{e}
Average daily gain (g/bird)	M + F	51^{a}	49^{b}	37^{c}	26^{e}	18^{e}
Average feed intake (g/bird and day)	M	76^{a}	71^{b}	60^{c}	42^{d}	30^{e}
	F	80^{a}	76^{a}	66^{b}	54^{c}	41^{d}
Average feed to gain ratio	M	1.50^{a}	1.53^{a}	1.77^{b}	1.95^{c}	2.34^{d}
	F	1.48^{a}	1.46^{a}	1.58^{b}	1.76^{c}	1.96^{d}
Haematology						
Eosinophils ($\times 10^3$/uL)	M	0.40	0.39	0.38	0.35	0.71
	F	0.52^{b}	0.98^{a}	1.14^{a}	0.35^{b}	0.18^{b}
Mean Corpuscular Haemoglobin (pg)	M	42.73^{a}	40.73^{ab}	38.13^{b}	38.35^{b}	38.15^{b}
	F	40.32	41.12	39.92	39.72	38.22
Serum chemistry						
AST (U/L serum)	M + F	184^{a}	243^{b}	250^{b}	245^{b}	235^{b}
Cholesterol (mg/dL serum)	M + F	129^{a}	137^{ab}	147^{bc}	154^{bc}	175^{c}
Calcium (mg/dL serum)	M + F	11.2^{a}	10.7^{ab}	10.2^{b}	10.4^{b}	10.2^{b}

nd: not detected.
Means in the same raw with different superscript are significantly different ($p \leq 0.05$).
(1): n out of 120 per treatment group including culled birds.

No statistically significant differences were observed in the haematological parameters between the different groups with the exception of increase absolute counts of eosinophils in females of the 1.25×, and a reduced MCH in males in lasalocid overdose groups (1.9×, 2.5× and 3.1×). The changes, however, were considered not biologically relevant.

Similarly, no statistically significant differences between treatment groups were observed for most clinical chemistry parameters.[23] A significantly increased of AST was observed in all four lasalocid groups (see Table A.1) compared to the control group. Significantly lower calcium levels were measured in the 1.9, 2.5 and 3.1× groups compared to the control group. The differences seen in these two parameters were not dose related. In contrast, total cholesterol increased significantly in a dose-dependent manner in the 1.9, 2.5 and 3.1× groups compared to the control. Differences seen between the three overdose groups and the control in other parameters (total protein, CK, GGT and serum potassium) were not considered biologically relevant due to small magnitude of the effects or effects only in one gender or a lack of a dose-dependent change.

Small differences were observed in the absolute organ weights of bursa of Fabricius, heart, kidneys, liver and spleen of the overdose groups and in bursa of Fabricius and kidneys (reduced weight) of the use level group compared to control birds. However, no differences in the relative weight of these organs were seen with the exception of bursa of Fabricius in which it was significantly reduced in the use level group compared to the control and the twofold overdose group. In summary, there were no relevant effects of the treatment on necropsy findings.

At the end of the experiment, the liver tissue from selected animals from the control group, the 1.25× and 1.9× overdose groups was microscopically examined. Liver tissue from 2.5× and 3.1× overdose groups was excluded from the evaluation.

[23] AP, ALT, blood urea nitrogen, chloride, glucose, lactate dehydrogenase, magnesium, sodium, phosphorus and uric acid.

Minimal to mild, panlobular hepatocellular hypertrophy was observed in the liver of lasalocid-treated groups. The incidence and severity of hepatocellular hypertrophy were slightly higher in the 1.9× overdose group compared to the 1.25× overdose. The hypertrophy was panlobular (expanding across the entire lobule) and was characterised by enlarged hepatocytes with hypereosinophilic to granular cytoplasm often with variable cytoplasmic clearing. At the 1.9× overdose, hypertrophy was occasionally accompanied by mild multifocal lymphohistiocytic infiltration (one animal) and/or increased numbers and size of Kupffer cells lining the sinusoids (hypertrophy/hyperplasia). No such findings were observed in the untreated control group.