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Carcinogenicity and chronic toxicity of copovidone (Kollidon VA 64) in Wistar rats and Beagle dogs

W. Mellert, K. Deckardt, C. Gembardt, B. Hildebrand, S. Schulte *

Product Safety—Regulations, Toxicology and Ecology, BASF Aktiengesellschaft, Carl-Bosch-Strasse 38, Ludwigshafen, Rheinland-Pfalz D67056, Germany

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Abstract

Kollidon VA 64 (copovidone, CAS-No. 25086-89-9) was administered in the diet to male and female Wistar rats (0, 700, 1400, and 2800 mg/kg body weight) for 24 months, and to male and female beagle dogs (0, 500, 1500, and 2500 mg/kg body weight/day) for 52 weeks. Clinical signs, body weight, food consumption, hematology, and gross and histopathological evaluations were conducted on both rats and dogs, and dogs also underwent hearing tests, ophthalmoscopic examinations, electrocardiograms, blood pressure measurement, and clinical chemistry and urinalysis evaluations. No adverse in-life observations related to treatment were observed in either species. The rats exhibited dark discoloration of the feces that was attributed to the intake and excretion of large amounts of test substance and was not considered to be an adverse effect. No treatment-related hematological changes, or gross or histopathological lesions were observed in either species that could be considered clinically relevant. Vacuolated histiocytosis in the mesenteric lymph nodes of four dogs that was not accompanied by inflammation or degenerative changes reflected histiocytic removal and degradation of the test article rather than a toxic effect. The results of these studies demonstrate the absence of any significant toxicological findings of high dietary levels copovidone in rats and dogs, resulting in no-observed-adverse-effect levels of 2800 mg/kg body weight/day in rats and 2500 mg/kg body weight/day in dogs, the highest doses tested.

Keywords: Copovidone; Copolyvidone; Copolymer; Vinylpyrrolidone; Carcinogenicity; Chronic toxicity; Rat; Beagle dog

1. Introduction

Kollidon VA 64 (copovidone, copolyvidone; CAS-No. 25086-89-9), is a copolymer of vinylpyrrolidone and vinyl acetate in a ratio of 6:4. It is a widely used excipient in the pharmaceutical industry, serving as a soluble binder and film-forming agent, particularly for solid dosage forms.

Copovidone is only minimally absorbed following oral administration (approximately 2% of the administered dose), and is, therefore, largely excreted in the feces (BASF unpublished data). The copolymer has demonstrated no acute toxicity and is not irritating to the skin or mucous membranes (BASF unpublished data). The chronic toxicity and carcinogenicity of copovidone were evaluated in Wistar rats in a 24-month feeding study, and its chronic toxicity was also investigated in a 52-week feeding study in beagle dogs. The results of these studies, which support the overall safety of this substance, are reported herein.

2. Materials and methods

2.1. 24-Month rat study

2.1.1. Test substance

Copovidone (>98% pure), a white-yellow powder, was provided by BASF.

2.1.2. Study design

Copovidone was administered in the diet to male and female Wistar rats at target dose levels of 0, 700, 1400, and 2800 mg/kg body weight over a period of 24 months. The actual doses received are indicated in

* Corresponding author. Tel.: +49-621-60-58042; fax: +49-621-60-58134.
E-mail address: stefan.schulte@basf-ag.de (S. Schulte).

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Table 1
Target and actual dose levels in 24-month rat study

Group	No. of animals/sex/dose			Target dose levels (mg/kg body weight/day)	Actual mean dose levels (mg/kg body weight/day)	
	Total	Main	Satellite		Males	Females
0	60	50	10	0	0	0
1	60	50	10	700	686	691
2	60	50	10	1400	1374	1378
3	60	50	10	2800	2625	2759

Table 1. Four main groups of 50 animals/sex/dose were included in the study. Additionally, four satellite groups, ($n = 10$ animals/sex/dose), were included for hematological evaluation at 6, 12, and 18 months. The test substance was administered daily in the diet for 24 months to the main groups and up to 18 months in the satellite groups. At the end of the administration period all animals were sacrificed after a 16–20-h fasting period.

This study followed OECD Guideline No. 451 (OECD, 1981a,b), as well as the requirements of the European Community Commission (1987) and US FDA (1982). The study was conducted in compliance with Good Laboratory Practice Standards.

2.1.3. Animals and maintenance

Male and female Wistar rats Chbb:THOM (SPF) were supplied by Dr. Karl Thomae GmbH, Biberach/Riss, FRG and acclimated for a period of 10 days. The animals were 42-days-old at the start of treatment. Mean body weights for the rats are provided in Table 4. Each animal was identified by an ear tattoo. The rats were housed singly in type DK III stainless steel wire mesh cages with absorbent dust free bedding (type 3/4, SSNIFF, Soest, FRG). Room temperature and relative humidity were maintained at 20–24 °C and 30–70%, respectively, with a photocycle of 12 h. Water and diet were supplied ad libitum.

2.1.4. Diet

The concentrations in the diet were adjusted weekly according to group mean body weight and food consumption to achieve the desired dose levels. The test substance was weighed out and thoroughly mixed with the corresponding amounts of food, depending on the dose group. The diet used was ground Kliba maintenance diet rat/mouse/hamster, 343 meal, supplied by Klingentalmühle AG, Kaiseraugst, Switzerland. The stability of the test substance in the diet at a concentration of approximately 0.2% was verified for 10 days at room temperature. As the mixtures were stored for no longer than 10 days, the stability of the diet mixture was ensured. The homogeneity of the mixtures was verified at a concentration of 55.000 ppm. The food used in the study was assayed for chemical as well as for microbiological contaminants and the levels were found to be under acceptable limits.

2.1.5. Observations

The animals were observed for clinical signs of toxicity or mortality at least once a day. Moreover, comprehensive clinical examinations and palpation of the animals were performed once a week. Food consumption, food efficiency, and body weight were determined once a week during the first 26 weeks, and thereafter at 4-week intervals.

2.1.6. Hematology

Blood samples were taken by tail vein puncture in the morning from non-fasted, unanesthetized animals. Hematological examinations were carried out in the satellite groups after about 6, 12, and 18 months, and in the main groups after 24 months. Hematological parameters included leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelets.

2.1.7. Necropsy and postmortem examination

All animals were killed by decapitation under CO_2 anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology. Animals that died intercurrently were necropsied as soon as possible after death and assessed by gross pathology.

2.1.8. Histopathology

Tissues collected for histopathological examination included all gross lesions, brain, pituitary gland, thyroid glands with parathyroid glands, thymus, trachea, lungs, heart, aorta, salivary glands (mandibular and sublingual glands), liver, spleen, kidneys, adrenal glands, pancreas, testes, ovaries, uterus (with cervix uteri/vagina/oviducts), epididymides, prostate gland, seminal vesicle, skin, esophagus, stomach (glandular and non-glandular), mandibular and mesenteric lymph nodes, female mammary gland, skeletal muscle, sciatic nerve, sternum with sternal bone marrow, bone marrow (femur), eyes with Harderian glands, femur with knee joint, spinal cord (cervical, thoracic, and lumbar cord), extraorbital lacrimal glands, and head with nasal cavities and Zym-

Table 2
Target and achieved dose levels in 52-week dog study

Group	No. animals/sex/dose	Target dose levels (mg/kg body weight/day)	Actual mean dose levels (mg/kg body weight/day)
1	6	0	0
2	4	500	510
3	4	1500	1518
4	6	2500	2522

bal glands. Tissues were fixed in 4% formaldehyde solution.

Histopathological examination was performed on all tissues from all animals in the control and high-dose groups from the main study. In addition, all gross lesions, the pituitary gland, thymus, lungs, liver, kidneys, testes, uterus, and female mammary gland were examined from all dose groups in the main study, and all tissues were examined in all dose groups from the satellite study. Tissues were embedded in paraffin, sectioned, and then stained with hematoxylin and eosin.

2.1.9. Statistical analyses

Food consumption, body weight, body weight change, and food efficiency were analyzed by parametric one-way analysis using the *F*-test (ANOVA, two-sided). If the resulting *p*-value was $\leqslant 0.05$, a comparison of each group with the control group using the Dunnett's test (two-sided) was performed for the hypothesis of equal means. Hematological parameters (except differential blood count) were analyzed by non-parametric one-way analysis using the Kruskal–Wallis test (two-sided). If the resulting *p*-value was $\leqslant 0.05$, a pairwise comparison of each dose group with the control group was performed using the Mann–Whitney *U*-test (two-sided for the equal medians).

2.2. 52-Week dog study

2.2.1. Test substance

Copovidone, a white-yellow powder meeting the specifications of the European Pharmacopoeia, was provided by BASF. The test substance's stability in the diet was verified for 5 days at room temperature and an additional 25 days when refrigerated. The moist test article/feed mixture (i.e., when mixed with an equal weight of water) was stable over 6 h. Intercurrent analyses for homogeneity, content, stability, and contaminants were conducted.

2.2.2. Study design

Copovidone was administered in the diet to male and female pure-bred beagle dogs over a period of 52 weeks. Animals were allocated to the following treatment groups: Group 1–0 mg/kg body weight/day (control) (six animals/sex); Group 2–500 mg/kg body weight/day (four animals/sex); Group 3–1500 mg/kg body weight/day (four animals/sex); and Group 4–2500 mg/kg body weight/day (six animals/sex). Additionally, at the end of the 52-week treatment period, two males and females each from Groups 1 and 4 (control and high-dose) were maintained treatment-free for an additional 6-week recovery period. The test substance was administered daily in the diet for 52 weeks. At the end of the administration period all animals were sacrificed by exsanguination following anesthetization with sodium pentobarbital. The doses received are indicated in Table 2.

This study followed OECD Guideline No. 452 (OECD, 1981a,b), as well as the requirements of the European Communities (Official Journal of the European Communities, 1975, 1983). The study was conducted in compliance with Good Laboratory Practice Standards.

2.2.3. Animals and maintenance

Male and female pure-bred beagle dogs were obtained from Marshall Farms USA, Inc. and acclimated for a period of 148 or 85 days under test conditions. The animals were 11–12-months-old at the start of treatment. Each animal was identified by an ear tattoo. The rats were housed in individual kennels. Room temperature and humidity were monitored continually, and kennel floor temperature was maintained at approximately 21 °C, with a photocycle of 12 h. Diet (350 g of dry feed mixed with 350 g water) was provided to the animals at 10:00 each morning and removed at 13:00. Water was supplied ad libitum.

2.2.4. Diet

The concentrations in the diet were adjusted weekly according to group mean body weight and food consumption to achieve the desired dose levels. The test substance was weighed out and thoroughly mixed with the corresponding amounts of food, depending on the dose group. The diet used was microgranulated standard Kliba dog maintenance diet supplied by KLIBA, Klingentalmühle AG, Kaiseraugst, Switzerland. The food used in the study was assayed for chemical as well as for microbiological contaminants and the levels were found to be under acceptable limits.

2.2.5. Observations

The animals were observed for clinical signs of toxicity or mortality at least twice per day. Food consumption was recorded daily, and summarized weekly. Body weight was determined once or twice a week throughout the study

2.2.6. Ophthalmoscopic examinations

Each animal was examined for abnormalities of the eyes (cornea, conjunctive, sclera, iris, lens, and fundus) following instillation of 0.5% tropicamide solution prior to the start of treatment, at 13, 26, and 51 weeks of treatment, and after the 6-week recovery period.

2.2.7. Hearing tests

Each animal was tested for hearing impairment using a simple noise test prior to the start of treatment, at 13, 26, and 51 weeks of treatment, and after the 6-week recovery period.

2.2.8. Electrocardiograms

Electrocardiograms (leads I, II, III, aVR, aVL, and aVF) of each animal were recorded using standard ECG methods prior to the start of treatment, at 13, 26, and 51 weeks of treatment, and after the 6-week recovery period. Heart rate, P wave duration and amplitude, and P–Q, QRS, and Q–T intervals were measured using a representative section from the Lead II ECG.

2.2.9. Blood pressure

Systolic arterial blood pressure was recorded by the tail-cuff method for each animal at 13, 26, and 51 weeks of treatment, and after the 6-week recovery period.

2.2.10. Hematology, clinical chemistry, and urinalysis

Blood and urine samples were taken from all animals between 6:00 and 7:40 in the morning following an overnight fast by jugular vein puncture at 13, 26, and 51 weeks of treatment, and after the 6-week recovery period.

Hematological parameters included leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and platelet count, reticulocyte count, reticulocyte fluorescence ratios, nucleated erythrocytes, total leukocyte count, differential leukocyte count, red cell morphology, and coagulation (thromboplastin time, activated partial thromboplastin time).

Clinical chemistry parameters included glucose, urea, creatinine, total bilirubin, total lipids, total cholesterol, triglycerides, phospholipids, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, glutamate dehydrogenase, creatine kinase, alkaline phosphatase, γ-glutamyl-transferase, iron, calcium, phosphorus, magnesium, sodium, potassium, chloride, total protein, and protein electrophoresis.

Urinalysis parameters included specific gravity, osmolality, color, appearance, pH, protein, glucose, ketones, bilirubin, blood, urobilinogen, and urine sediment.

2.2.11. Necropsy and postmortem examination

All animals were anesthetized by intravenous injection of sodium pentobarbital and killed by exsanguination. The exsanguinated animals were necropsied and assessed by gross pathology.

2.2.12. Organ weights

The following organ weights were recorded prior to fixation: adrenal glands, brain (including brain stem), heart, kidneys, liver, pituitary gland, prostate gland, spleen, testes with epididymides, and thyroid gland with parathyroid.

2.2.13. Histopathology

Histopathological examination was performed on the following tissues from all animals: adrenal glands, aorta, bone (femur including articular surface), bone marrow (femur and sternum), brain (including medulla/pons, cerebral and cerebellar cortex), epididymides, esophagus, eyes with optic nerve, gallbladder, heart, kidneys, large intestine (cecum, colon, and rectum), larynx, liver, lungs infused with formaldehyde solution, lymph nodes (mesenteric and retropharyngeal), mammary gland area, ovaries, pancreas, pituitary gland, prostate gland, salivary glands (parotid, mandibular, and sublingual), sciatic nerve, skeletal muscle, skin, small intestine (duodenum, jejunum, and ileum), spinal cord (cervical, midthoracic, and lumbar segments), spleen, stomach, testes, thymus, thyroid gland (including parathyroid gland), tongue, trachea, urinary bladder, uterus with vagina, and all gross lesions.

Tissues were fixed in neutral phosphate-buffered 4% formaldehyde solution. The eyes with optic nerve were fixed in Heidenhain's Susa solution, and bone marrow smears from one sternebrum from all animals were taken for possible future examination. Tissues were embedded in paraffin, sectioned, and then stained with hematoxylin and eosin.

2.2.14. Statistical analyses

Food consumption, body weight, organ weights, electrocardiograms, blood pressure, and clinical laboratory data were analyzed by univariate one-way analysis of variance (ANOVA). If the variables could be assumed to follow a normal distribution, the Dunnett-test (many-one *t*-test) based on a pooled variance estimate was applied for the comparison between the treated groups and the control groups. The Steel-test (many-one rank test) was applied when the data could

not be assumed to follow a normal distribution. Group means were calculated for continuous data and medians were calculated for discrete data (scores) in the summary tables.

3. Results

3.1. 24-Month rat study

3.1.1. In-life observations

Mortality rates for all groups are provided in Table 3. The mortality rates ranged from 14% in the high-dose males to 36% in the control males, and 26% in the high-dose females to 30% in the control females. Comparable data were obtained in the satellite groups (data not shown). Based on the lack of a dose–response relationship, and a higher mortality rate in the control animals of both sexes, it can be concluded that copovidone did not affect survival.

Dark discoloration of the feces was observed in all treated animals. This finding, observed in previous studies, was attributed to the intake and excretion of large amounts of test substance and was not considered to be an adverse effect (BASF, unpublished data). Additional examinations of the feces hemoglobin content with benzidine revealed that there was no blood in the stool, and confirmed the lack of an adverse effect.

Food consumption was normal, with the exception of one day in which the high concentration of copovidone (2800 mg/kg, approximately 65.000 ppm) made the food unpalatable. The concentration of test substance was thereafter fixed at 55.000 ppm in this group for the duration of the study. No other substance-related clinical signs were observed. A few isolated statistically significant differences in different test groups during the study were observed. For example, although mid-dose females showed periods of decreased consumption (days 119, 133, 147, 154, 161, 168, and 602), high-dose females showed both increased (days 175, 238, and 266) and decreased (days 133 and 168) consumption on various days. Because of their incidental nature, isolated occurrence, and lack of a dose–response relationship, these deviations were considered not to be treatment-related. Food efficiency varied significantly in a few sporadic incidences between groups, but the changes were not dose-related and determined to be incidental.

Table 3
Cumulative mortality in 24-month rat study (main groups, 50 animals per dose and sex)

Group	Dose (mg/kg)	Mortality *N* (%)	
		Males	Females
0	0	18 (36%)	15 (30%)
1	700	18 (36%)	15 (30%)
2	1400	7 (14%)	14 (28%)
3	2800	7 (14%)	13 (26%)

Body weight and body weight change were statistically significantly reduced in the high-dose males at most time points throughout most of the study, resulting in reduced values of 9.5% and 12.1% below controls on day 728, respectively (see Table 4). This change was considered to be treatment-related. In contrast, in the low-dose males, there were only sporadic statistically significant decreases in body weight (days 686 and 714) and body weight change (day 686). Because of the isolated nature of these changes, and the lack of significant changes in the mid-dose males, the body weight decreases in low-dose males were considered to be incidental in nature, and not treatment-related. Aside from increased body weight in low-dose females on days 462 and 490, increased body weight change in low-dose females on days 462, 490, and 574, and decreased body weight change in high dose females on days 168 and 714, there were no statistically significant differences in body weight and body weight change between treated and control females. Due to the lack of a sustained, dose-dependent relationship, these isolated instances of body weight differences were likewise determined not to be related to treatment. Changes in the body weights of the high-dose satellite males were more pronounced than in the main group; however, these differences were not statistically significant (data not shown).

3.1.2. Hematology

The following statistically significant changes in hematological parameters were observed: white blood cells—high-dose males at Day 176; low- and mid-dose females at Day 543; red blood cells—high-dose males at Day 176; mean corpuscular volume—low- and high-dose males at Day 725; mean corpuscular hemoglobin—high-dose males at Day 725; and mean corpuscular hemoglobin concentration—mid-dose males at Day 725. These differences were marginal, within historical control ranges, inconsistent across time periods and sex, and/or did not demonstrate a dose–response relationship, and therefore, are not considered treatment-related.

3.1.3. Gross pathology

Gross lesions were found in various parenchymatous organs (liver, lungs, kidneys, and heart); endocrine organs (adrenal cortex and pituitary gland); gastrointestinal tract (glandular stomach); immune competent organs (spleen, thymus); the genital tract of male (testes) and female animals (ovaries, cervix uteri); and a variety of other organs (e.g., urinary bladder, mammary gland, brain, eyes, and skin (see Table 5).

The vast majority of gross lesions in the main groups occurred with a comparable incidence to the controls, and/or with no clear dose–response relationship.

Table 4
Body weight and body weight change in 24-month rat study (main groups)

Group	Value	Body weight (g)					
		Males			Females		
		Day 0	Day 350	Day 728	Day 0	Day 350	Day 728
0 (0 mg/kg)	Mean	187.9	664.7	710.9	142.5	332.2	397.1
	% Control	100.0	100.0	100.0	100.0	100.0	100.0
	SD	7.9	78.3	125.3	5.9	25.5	58.0
	N	50	48	33	50	50	35
1 (700 mg/kg)	Mean	186.1	643.3	663.3	142.9	339.7	396.5
	% Control	99.0	96.8	93.3	100.3	102.3	99.9
	SD	7.6	64.5	96.2	6.2	28.4	78.1
	N	50	49	32	50	50	35
2 (1400 mg/kg)	Mean	184.9	635.5	678.8	143.7	330.8	379.1
	% Control	98.4	95.6	95.5	100.8	99.6	95.5
	SD	8.7	68.4	92.3	5.4	29.2	43.8
	N	50	49	43	50	50	36
3 (2800 mg/kg)	Mean	184.7	617.2**	643.1**	142.8	322.6	370.3
	% Control	98.3	92.9	90.5	100.2	97.1	93.3
	SD	7.6	60.6	72.4	5.6	24.2	62.2
	N	50	50	44	50	50	38
		Day 7	Day 350	Day 728	Day 7	Day 350	Day 728
0 (0 mg/kg)	Mean	54.5	476.5	521.8	24.8	189.7	254.8
	% Control	100.0	100.0	100.0	100.0	100.0	100.0
	SD	5.2	74.9	125.4	4.2	22.9	59.1
	N	50	48	33	50	50	35
1 (700 mg/kg)	Mean	55.6	457.0	477.8	26.2	196.8	254.1
	% Control	102.0	95.9	91.6	105.4	103.7	99.7
	SD	7.0	62.6	95.9	4.7	26.1	76.6
	N	50	49	32	50	50	35
2 (1400 mg/kg)	Mean	54.6	450.7	493.4	24.6	187.1	235.5
	% Control	100.2	94.6	94.6	99.2	98.6	92.5
	SD	8.8	65.4	89.3	5.5	26.9	42.1
	N	50	49	43	50	50	36
3 (2800 mg/kg)	Mean	51.6	432.5**	458.5*	24.9	179.9	228.2
	% Control	94.7	90.8	87.9	100.3	94.8	89.6
	SD	8.4	57.1	71.4	3.9	21.0	61.1
	N	50	50	44	50	50	38

Statistics: Anova + Dunnett's test (two-sided): $^{*}P \leqslant 0.05$ $^{**}P \leqslant 0.01$.

3.1.4. Histopathology

In general, most of gross lesions noted could be correlated with a meaningful microscopic finding. Table 6 summarizes all primary tumors seen more often than once or twice in control or high dose groups, respectively. There was no treatment-related increase in the number of animals with: neoplasm; one or more than one primary neoplasm; or benign, malignant, systemic, and metastasized neoplasms. Moreover, there was no treatment-related increase in the total number of primary neoplasms, or benign, malignant, systemic, or metastasized neoplasms (see Table 7). Finally, there was no indication that the test article resulted in any non-neoplastic alteration of organs or organ systems when comparing the incidence and graded severity of microscopic findings of treated animals with the corresponding observations of control animals (see Table 8). All neoplastic and non-neoplastic microscopic findings were considered to have developed spontaneously and were unrelated to treatment.

3.2. 52-Week dog study

3.2.1. In-life observations

No animals died during the course of the study, and no treatment-related clinical signs were observed. Food consumption, ophthalmoscopic examinations, hearing tests, electrocardiograms, and blood pressure were similarly unaffected by treatment with copovidone.

Slight, but persistent, weight loss was recorded in two males (numbers 15 and 20) from Group 4 (2500 mg/kg), culminating in a difference of 1.1–1.2 kg at the end of the

Table 5
Incidence of relevant gross lesions in 24-month rat study

Sex	Male				Female			
Dose group	0	1	2	3	0	1	2	3
Animals in selected group	50	50	50	50	50	50	50	50
Adrenal cortex								
Enlarged	1	–	–	1	3	5	2	6
Focus	11	11	19	12	39	44	41	41
Brain								
Compression	–	–	4	1	17	16	17	12
Cervix								
Induration	–	–	–	–	2	6	5	6
Mass	–	–	–	–	–	3	6	1
Eyes								
Cataract	2	6	3	1	–	2	2	3
Glandular stomach	–	–	–	–	–	–	–	–
Erosion/ulcer	10	13	9	6	6	6	5	9
Heart								
Calcification	2	–	–	–	2	1	5	–
Dilation	5	1	1	4	–	2	3	–
Kidneys								
Cyst	2	5	4	1	3	–	1	–
Granular surface	9	8	12	6	3	1	6	7
Retraction	3	8	2	9	1	2	5	3
Liver								
Cyst	9	4	10	12	11	13	11	10
Focus	32	37	43	41	29	22	23	25
Mass	5	6	5	2	–	4	1	3
Lungs								
Discoloration	5	5	1	5	5	2	3	2
Focus	6	8	11	7	1	4	1	2
Mammary gland								
Mass	1	1	–	–	8	14	12	14
Ovaries								
Cyst	–	–	–	–	13	17	13	8
Pituitary gland								
Enlarged	3	–	1	1	5	6	3	1
Focus	4	5	2	5	15	14	10	11
Mass	1	4	10	6	25	23	28	24
Seminal vesicle								
Organ size reduced	5	7	3	3	–	–	–	–
Skin								
Decubitus	6	7	8	3	1	1	–	–
Mass	6	2	6	5	–	1	1	1
Spleen								
Mass	1	5	1	2	–	–	–	–
Testes								
Calcification	8	4	5	6	–	–	–	–
Cystic degeneration	8	6	6	9	–	–	–	–
Focus	6	6	10	6	–	–	–	–
Mass	6	11	17	15	–	–	–	–
Organ size reduced	2	4	2	5	–	–	–	–
Thymus								
Mass	4	7	3	3	1	1	3	4
Urinary bladder								
Dilation	6	1	1	–	–	–	1	–

treatment period. Neither animal gained weight during the recovery period. These body weight losses are unlikely to be related to treatment, as one of the dogs exhibited a similar trend prior to study commencement, and the remaining male and female dogs of this treatment group exhibited weight gains similar to those of the controls.

3.2.2. Hematology, clinical chemistry, and urinalysis

Hematology, clinical biochemistry and urinalysis parameters were unaffected by the administration of copovidone. Although sporadic, statistically significant intergroup differences were seen, these did not exhibit any dose-related or time-related pattern, and were not

Table 6
Incidence of relevant primary neoplasms in 24-month rat study

Sex	Male				Female			
Dose group	0	1	2	3	0	1	2	3
Animals in selected group	50	50	50	50	50	50	50	50
Adrenal medulla	50	30	28	50	50	49	49	50
Pheochromocytoma, benign	7	5	9	11	2	5	4	2
Pheochromocytoma, malignant	2	1	–	1	–	1	–	–
Brain	50	19	10	50	50	26	25	50
Granular cell tumor	–	–	1	2	–	–	–	1
Female mammary gland	1	2	–	–	50	50	49	49
Fibroadenoma	1	–	–	–	5	8	8	9
Adenoma	–	–	–	–	3	1	–	–
Adenocarcinoma	–	1	–	–	2	5	4	4
Heart	50	18	9	50	50	18	19	50
Schwannoma, endoc. benign	3	–	–	–	–	–	1	–
Hemolymphatic system	50	18	8	50	50	15	14	50
Malignant lymphoma	2	1	2	2	1	–	–	1
Sarcoma, histiocytic	1	5	1	–	–	1	–	2
Liver	50	50	50	50	50	50	50	50
Adenoma, hepatocellular	5	4	3	2	3	1	–	1
Carcinoma, hepatocellular	2	1	2	1	–	1	1	1
Mesenteric lymph nodes	50	20	8	50	50	15	14	50
Lymphangioma	1	2	–	1	1	1	–	–
Hemangioma	7	1	–	6	–	–	–	–
Hemangiosarcoma	1	2	1	–	–	–	–	1
Ovaries	–	–	–	–	50	34	27	50
Tumor, granular cell benign	–	–	–	–	5	–	–	2
Pituitary gland	50	50	50	50	50	50	50	50
Adenoma, pars dist.	5	7	12	8	22	22	28	19
Carcinoma, pars dist.	–	–	–	1	5	4	1	2
Skeletal muscle	50	18	7	50	50	15	14	50
Schwannoma, malignant	2	–	–	–	–	–	–	–
Skin	50	26	18	50	50	16	15	50
Tumor, hair follicle	–	–	–	2	–	–	–	–
Keratoacanthoma	–	1	4	–	1	–	1	–
Fibroma	2	1	–	1	–	–	–	–
Schwannoma, malignant	2	–	–	–	–	–	–	–
Spleen	50	20	11	50	50	17	16	50
Hemangiosarcoma	2	4	3	2	–	–	1	–
Testes	50	50	50	50	–	–	–	–
Tumor, Leydig cell benign	13	19	24	20	–	–	–	–
Thymus	50	50	50	50	50	50	50	50
Thymoma, benign	4	8	4	3	4	2	3	4
Thyroid glands	50	18	8	50	50	16	16	50
Tumor, C-cell, benign	4	2	2	7	8	2	1	9
Uterus	–	–	–	–	50	50	50	50
Polyp, stromal	–	–	–	–	6	6	4	5

consistently present for both sexes. Therefore, the differences in these parameters are considered to represent the expected spontaneous variations that occur in dogs of this strain and age.

3.2.3. Organ weights

Organ weights and organ to body weight ratios were unaffected by treatment with copovidone. The only statistically significant difference observed was a slight decrease in thyroid weight in the high-dose males. This effect was not dose-related, and was not observed in the females.

3.2.4. Gross pathology

A number of macroscopic findings were observed in dogs of all groups. The type and incidence of these

Table 7
List of tumor bearing animals and summary of tumors in 24-month rat study

Sex	Male				Female			
Dose group	0	1	2	3	0	1	2	3
Animals in selected group	50	50	50	50	50	50	50	50
Number of animals with:								
Neoplasms	44	44	43	42	41	42	44	42
1 Primary neoplasms	23	22	21	16	17	21	22	18
2 Primary neoplasms	12	15	15	16	15	14	16	17
3 Primary neoplasms	6	7	6	9	7	7	6	7
4 Primary neoplasms	2	–	1	1	2	–	–	–
5 Primary neoplasms	1	–	–	–	–	–	–	–
Number of animals with:								
Benign neoplasms	35	38	39	40	36	34	37	36
Benign neoplasms only	25	24	33	31	29	26	29	28
Malignant neoplasms	19	20	10	11	12	16	15	14
Malignant neoplasms only	9	6	4	2	5	8	7	6
Systemic neoplasms	3	6	3	2	1	1	–	3
Metastasized neoplasms	5	6	2	1	–	–	–	1
Total number of:								
Primary neoplasms	78	73	73	79	76	70	72	73
Benign neoplasms	57	53	61	67	63	52	55	57
Malignant neoplasms	21	20	12	12	13	18	17	16
Systemic neoplasms	3	6	3	2	1	1	–	3
Metastasized neoplasms	5	6	3	1	–	–	–	1

macroscopic findings were comparable across treated and control groups.

3.2.5. Histopathology

A number of microscopic findings were observed in dogs of both treated and control groups (see Table 9). Except for the vacuolated histiocytosis in a mesenteric lymph node of two dogs in the high-dose group (19 M and 39 F), and one female each from the low- and mid-dose groups, the incidence and severity of these findings were comparable across treated and control animals, and are commonly observed changes in dogs of this age and strain.

In these animals, minimal to moderate numbers of vacuolated histiocytes were diagnosed in the sinusoids and trabeculae of some mesenteric lymph nodes. The cytoplasm of these histiocytes contained either large clear vacuoles or were foamy in appearance with a slight basophilic tinge. This change was termed "vacuolated histiocytes, sinusoidal and trabecular." This change was moderate in the two dogs in the high-dose group, slight in the mid-dose female, and minimal in the low-dose female. No inflammatory and/or degenerative changes (i.e., necrosis, granulomas, etc.) were associated with vacuolated histiocytes.

When the mesenteric lymph nodes were reacted with chlorazol-fast-pink (a test for polyvinyl pyrrolidones), positive staining histiocytes were observed in some dogs of all treated groups after 52-weeks of treatment (four females of Group 4, three males of Group 3, and one male of Group 2), and in two females of Group 4 at the end of the 6-week recovery period. There were no

Table 8
Incidence of relevant non-neoplastic findings in 24-month rat study

Sex	Male				Female			
Dose group	0	1	2	3	0	1	2	3
Animals in selected group	50	50	50	50	50	50	50	50
Adrenal cortex	50	31	28	50	50	49	49	50
Extracortical nodule	9	2	8	5	3	5	3	6
Blood filled cyst(s)	17	17	15	13	50	45	45	47
Hematopoiesis	3	1	1	1	2	7	3	4
Fatty focus/foci	9	2	7	15	5	6	5	2
Focal hypertrophy	4	3	6	3	9	6	6	11
Focal hyperplasia	21	5	7	19	24	19	21	19
Atrophy	4	4	3	4	5	8	9	7
Adrenal medulla	50	30	28	50	50	49	49	50
Hyperplasia, focal	13	7	1	12	11	8	13	11
Brain	50	19	10	50	50	26	25	50
Compression, focal	–	–	3	2	17	15	15	9
Hemorrhage(s)	–	1	–	1	7	4	2	3
Dilation, ventricle	2	4	1	5	11	3	4	8
Cervical cord	50	18	7	50	49	15	14	49
Calcification, meninx	2	–	–	7	1	–	–	–
Cervix uteri	–	–	–	–	50	50	50	50
Fibrosis	–	–	–	–	2	9	1	3
Coagulation glands	49	20	10	50	–	–	–	–
Involution/atrophy	6	5	2	5	–	–	–	–
Epididymides	50	20	9	50	–	–	–	–
Debris in lumen	7	3	2	7	–	–	–	–
Oligospermia	5	1	1	7	–	–	–	–
Azoospermia	8	4	–	12	–	–	–	–
Extraorbital lacr. glands	50	18	7	50	50	15	14	50
Chronic inflammation	44	14	3	48	20	4	5	19
Eyes	50	22	11	50	50	16	15	50
Lenticular degenerat	17	14	7	6	8	9	7	8
Female mammary glands	1	2	–	–	50	50	49	49
Lacteal cyst(s)	–	–	–	–	7	10	15	6
Hyperplasia, focal	–	–	–	–	7	5	10	8
Hyperplasia, diffuse	–	–	–	–	8	6	7	7
Glandular stomach	50	21	12	50	50	19	17	50
Erosion(s), focal	6	10	6	4	6	6	5	8
Harderian glands	50	22	11	50	50	16	15	50
Inflammation, focal	30	12	5	44	34	6	6	37
Heart	50	18	9	50	50	18	19	50
Dilation, ventricle	11	2	4	2	–	3	3	–
Myocardial fibrosis	41	14	8	43	35	9	10	25
Metaplasia, endocard	4	1	1	3	3	3	4	9
Kidneys	50	50	50	50	50	50	50	50
Cortical cyst(s)	2	6	1	1	3	1	3	5
Lymyphoid cell infiltr.	9	9	11	12	20	21	15	18
Microabscess(es)	11	19	16	18	14	14	14	20
Proteinaceous casts	10	9	10	13	23	21	18	21
Chronic nephropathy	33	37	37	34	19	21	26	20
Calcification, cortex	8	8	9	5	50	50	49	46
Calcification, pelvis	17	11	18	25	27	29	32	32
Pyelitis	6	2	2	2	1	1	–	–
Hyperplasia uroth. focal	13	13	19	9	14	25	20	18
Hyperplasia uroth. diffuse	9	3	2	2	1	–	1	1
Liver	50	50	50	50	50	50	50	50
Focal fatty infiltr.	31	23	30	33	19	23	27	17
Periph. fatty infiltr.	16	13	12	8	15	10	15	6
Hemosiderin desposit	6	3	4	8	1	2	2	–
Lymphoid cell infiltr.	32	31	41	44	38	39	39	35
Hematopoiesis	9	8	4	5	9	11	16	15
Focal peliosis	13	5	4	13	10	8	6	9
Spongiosis hepatis	20	23	27	14	1	4	2	2
Altered focus/foci	28	32	38	37	30	26	33	24
Clear cell focus	20	24	32	30	11	13	16	7

Table 8 (*continued*)

Sex	Male				Female			
Dose group	0	1	2	3	0	1	2	3
Animals in selected group	50	50	50	50	50	50	50	50
Eosinophilic focus	–	1	–	3	3	3	4	3
Basophilic focus	25	30	33	31	25	24	30	18
Biliary cyst(s)	12	5	8	7	13	13	10	11
Bile duct proliferation	24	30	37	31	32	35	34	30
Dystrophy	5	9	3	2	–	3	4	2
Lungs	50	50	50	50	50	50	50	50
Foam cell aggregation	19	21	24	17	7	8	8	7
Edema	9	3	2	3	7	1	2	2
Chronic pneumonitis	5	4	7	7	2	4	7	3
Mandibular lymph nodes	50	18	8	50	50	15	14	50
Hemosiderin deposit.	19	4	–	21	26	7	5	25
Pigment deposition	3	–	1	3	4	4	1	7
Hyperplasia plasma cells	40	12	5	40	43	12	14	38
Mesenteric lymph nodes	50	20	8	50	50	15	14	50
Blood resorption	11	4	2	8	2	–	1	2
Histiocytosis	43	14	6	47	47	14	14	47
Lymphoid hyperplasia	5	2	2	2	20	–	1	19
Hyperplasia, angiomat.	8	1	–	5	–	1	–	–
Ovaries	–	–	–	–	50	34	27	50
Cystic bursa ovarica	–	–	–	–	3	8	9	3
Follicle(s) present	–	–	–	–	27	25	17	20
Cystic follicle(s)	–	–	–	–	22	15	13	27
Corpus luteum present	–	–	–	–	18	12	9	17
Cyst(s)	–	–	–	–	30	19	12	23
Hyperplasia, sex c.	–	–	–	–	41	27	18	36
Pancreas	49	20	10	50	50	16	14	50
Exocrine atrophy	6	3	1	9	–	2	1	1
Parathyroid glands	48	17	8	49	47	16	16	47
Hyperplasia	8	4	1	6	1	1	–	1
Pituitary gland	50	50	50	50	50	50	50	50
Cyst(s), pars distal.	11	10	14	10	23	29	34	32
Cyst(s), pars interm	20	17	14	20	8	5	7	4
Hyperplasia, pars distal.	8	9	9	1	8	10	6	7
Prostate gland	50	21	8	50	–	–	–	–
Inflammation	13	7	6	13	–	–	–	–
Hyperplasia, focal	18	4	–	12	–	–	–	–
Sciatic nerve	50	19	7	50	50	15	14	50
Fiber degeneration	25	4	2	31	34	5	8	32
Seminal vesicle	50	21	10	50	–	–	–	–
Involution/atrophy	10	8	3	6	–	–	–	–
Skin	50	26	18	50	50	16	15	50
Hyperkeratosis, focal	6	7	7	5	1	1	–	–
Acanthosis, focal	7	7	7	4	1	1	–	–
Chronic inflammation	6	5	7	4	1	3	1	2
Ulceration, focal	6	5	6	4	1	1	–	–
Spleen	50	20	11	50	50	17	16	50
Hemosiderin deposit	41	10	8	45	49	14	13	47
Lymphocyte depletion	8	5	3	–	2	2	4	7
Testes	50	50	50	50	–	–	–	–
Edema, interstitial	11	6	5	9	–	–	–	–
Foc. tubular atrophy	24	26	25	23	–	–	–	–
Diff. tubular atrophy	11	9	9	14	–	–	–	–
Tubular dilat., focal	6	3	6	15	–	–	–	–
Calcification, tubuli	14	8	16	15	–	–	–	–
Leydig cell hyp., focal	32	27	25	31	–	–	–	–
Thoracic cord	50	18	7	50	50	15	14	50
Calcification, meninx	7	1	–	11	5	–	–	2
Thymus	50	5	50	50	50	50	50	50
Cyst(s)/cystic ducts	–	1	–	–	12	12	11	8
Thyroid glands	50	18	8	50	50	16	16	50
C-cell hyerplasia, d.	33	7	1	37	36	9	8	33

(*continued on next page*)

Table 8 (*continued*)

Sex	Male				Female			
Dose group	0	1	2	3	0	1	2	3
Animals in selected group	50	50	50	50	50	50	50	50
Urinary bladder	50	19	7	50	50	15	14	50
Cystitis	3	–	–	6	1	–	–	–
Lymphoid cell infiltr.	9	–	1	11	3	–	–	2
Hyperplasia, trans.	8	–	2	10	1	2	–	1
Uterus	–	–	–	–	50	50	50	50
Dilatation	–	–	–	–	5	12	11	4
Glandular cyst(s)	–	–	–	–	9	3	3	6
Vagina	–	–	–	–	50	50	50	50
Inflammation	–	–	–	–	12	9	9	7

inflammatory or degenerative changes associated with these findings. Therefore, these changes are considered not to represent a toxic effect of the test article, but reflect a successful attempt of the histiocytes of the mesenteric lymph nodes to remove and possibly degrade the test article.

4. Discussion

The results of both the chronic/carcinogenicity study in rats and the chronic toxicity study in dogs demonstrate that copovidone is not toxic to rats or dogs at dietary levels as high as 2800 mg/kg body weight per day (for rats) or 2500 mg/kg body weight/day (for dogs). Moreover, the rat study provided no evidence of a carcinogenic effect related to treatment with copovidone.

In the rat study, the high-dose males exhibited a statistically significant reduction in body weight and body weight change. This effect was not accompanied by any other treatment-related change in clinical signs, hematology, or pathology. Hematology parameters exhibited occasional statistically significant differences from control values, but these were judged to be incidental, and all values were within historical control ranges.

A dark discoloration of the feces in the rat study was seen in all treatment groups. This finding was considered to be a consequence of the intake and excretion of large amounts of the test substance rather than being an adverse effect. This assessment was confirmed by additional examinations of the feces for hemoglobin using benzidine, which were negative. Discoloration of the feces is therefore not caused by bleeding into the gastrointestinal tract, and thus is not considered an adverse effect.

There was no treatment-related increase in the number of animals with neoplasms; one or more than one primary neoplasm; benign, malignant, systemic, and metastasized neoplasms; or the total number of primary neoplasms; and benign, malignant, systemic, and metastasized neoplasms. Moreover, there was no indication that copovidone administration was associated with any non-neoplastic change in any of the organs or organ systems examined when the incidence and graded severity of the gross and microscopic findings of the treated animals were compared to their corresponding control group. All neoplastic and non-neoplastic findings were considered to have developed spontaneously and were unrelated to treatment.

In the dog study, in-life observations, hematology, clinical chemistry, and urinalysis demonstrated no clinically relevant changes that could be considered related to copovidone administration. The occasional differences seen in these parameters are considered to be the result of spontaneous variations that occur in dogs of this strain and age.

Minimal to moderate vacuolated histiocytosis in the mesenteric lymph nodes of one dog each at 500 or 1500 mg/kg body weight/day and two dogs at 2500 mg/kg body weight/day. This change was considered to be related to the administration of copovidone in the diet. When the mesenteric lymph nodes were reacted with chlorazol-fast-pink (a test for polyvinylpyrrolidones), positive staining histiocytes were observed in some dogs of all treated groups after 52 weeks of treatment and in two females at 2500 mg/kg body weight/day at the end of the recovery period. There were no inflammatory or degenerative changes associated with these findings. Therefore, it has been concluded that these changes were not a toxic effect of copovidone but, rather, reflect a successful attempt of the histiocytes of the mesenteric lymph nodes to remove and possible degrade the test article.

Based on the findings of these two studies, 2800 mg/kg body weight/day can be considered the no-observed-adverse-effect level ("NOAEL") for copovidone in rats and 2500 mg/kg body weight/day in dogs under the test conditions applied.

The results of these studies demonstrate the absence of any significant toxicological findings of copovidone in rats and dogs even at very high dose levels and support the safety of the compound for long-term oral use in humans.

Table 9
Incidence of relevant microscopic findings in 52-week dog study (main groups)

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Animals in selected group	4	4	4	4	4	4	4	4
Adrenal glands	4	4	4	4	4	4	4	4
Cytopl. vacuolation	–	–	1	1	2	2	3	2
Capsular cyst(s)	–	–	–	1	–	–	–	–
Focal hypertrophy	–	–	–	1	–	–	–	–
Focal mineralization	–	–	–	–	1	–	–	–
Cecum	–	–	–	–	4	4	4	4
Congestion	–	–	–	–	–	1	–	–
Colon	–	–	–	–	4	4	4	4
Congestion	–	–	–	–	–	1	–	–
Duodenum	4	4	4	4	4	4	4	4
Congestion	1	–	–	1	2	1	1	2
Distended glands	1	1	2	4	–	2	2	2
Esophagus	4	4	4	4	4	4	4	4
Lymphoid c. infiltr.	–	–	1	–	1	2	1	1
Gall bladder	4	4	4	4	–	–	–	–
Cyst. Mucosal Change	–	–	–	1	–	–	–	–
Heart	4	4	4	4	4	4	4	4
Inflammatory c. focus	1	1	1	–	1	2	–	–
Focal myodegeneration	–	–	–	–	–	1	–	–
Ileum	–	–	–	–	4	4	4	4
Congestion	–	–	–	–	–	1	–	–
Jejunum	–	–	–	–	4	4	4	4
Congestion	–	–	–	–	–	1	–	–
Kidneys	4	4	4	4	4	4	4	4
Lymphoid c. infiltr.	–	–	–	–	3	1	–	1
Medul. mineralization	4	4	4	4	4	4	4	4
Cort. mineralization	–	–	–	–	1	–	–	1
Lipid glomerulopathy	–	–	–	–	1	–	–	–
Infarcts	–	–	–	–	1	–	–	–
Pyelitis	–	–	–	–	–	1	–	–
Focal papillitis	–	–	–	–	–	1	–	–
Liver	4	4	4	4	4	4	4	4
Inflammatory c. foci	4	4	4	1	4	4	4	4
Diffuse fatty change	–	–	1	–	–	–	1	–
Fatty change/zone 3	–	–	–	–	1	1	–	–
Granulocytosis	–	–	–	–	–	1	–	–
Lung	4	4	4	4	4	4	4	4
Perivas. lymph. infilt.	1	–	2	1	–	3	–	–
Alveo. histiocytosis	–	–	2	–	–	2	2	2
Alveo. hyperplasia	–	–	1	–	–	–	1	–
Interstit. fibrosis	–	–	1	–	–	–	1	1
Focal bronchiolitis	–	–	–	1	–	–	–	–
Focal pneumonia	–	–	–	–	–	–	–	1
Lymph nodes	1	–	–	1	2	–	1	–
Congestion	1	–	–	1	2	–	–	–
Medulla oblongata	–	–	–	–	4	4	4	4
Perivascular cuffing	–	–	–	–	–	1	–	–
Mesenteric lymph nodes	4	4	4	4	4	4	4	4
Congestion	1	3	2	2	1	2	1	2
Lymphoid hyperplasia	–	–	–	–	–	–	–	1
Vacuolat. histiocytos.	–	–	–	1	–	1	1	1
Mesenteric lymph nodes/CFP	4	4	4	4	4	4	4	4
PVP positive cells	–	1	3	–	–	–	–	4
Ovaries	–	–	–	–	4	4	4	4
Normal corpora lutea	–	–	–	–	1	–	–	–
Cyst(s)	–	–	–	–	–	1	–	–
Congestion	–	–	–	–	–	1	–	2
Parathyroid glands	4	4	4	4	4	4	4	4
Cyst(s)	1	2	–	–	–	–	–	1
Parotid gland	4	4	4	4	4	4	4	4

(continued on next page)

Table 9 (*continued*)

Sex	Male				Female			
Dose group	1	2	3	4	1	2	3	4
Animals in selected group	4	4	4	4	4	4	4	4
Lymphoid c. infiltr.	2	3	2	1	2	–	1	–
Focal atrophy	–	–	–	–	–	–	–	1
Pituitary gland	4	4	4	4	4	4	4	4
Cyst(s)	2	1	2	–	–	1	2	–
Prostate gland	4	4	4	4	–	–	–	–
Inflammation	3	–	2	2	–	–	–	–
Cystic change	1	2	2	–	–	–	–	–
Focal atrophy	2	1	2	–	–	–	–	–
Retropharyngeal lnn.	4	4	4	4	4	4	4	4
Congestion	1	–	1	–	–	1	1	–
Tattoo ink	1	2	–	–	–	–	–	–
Erythrophagocytosis	–	–	–	–	–	1	–	–
Sinus histiocytosis	–	–	–	–	–	1	–	–
Skin/subcutis	–	–	–	–	4	4	4	4
Follicular keratosis	–	–	–	–	1	–	–	–
Epithel. hyperplasia	–	–	–	–	1	–	–	–
Hyperkeratosis	–	–	–	–	1	–	–	–
Dermatitis	–	–	–	–	1	–	–	–
Epidermitis	–	–	–	–	1	–	–	–
Spleen	4	4	4	4	4	4	4	4
Congestion	1	–	–	1	1	2	–	1
Siderotic plague	1	–	1	–	–	–	–	–
Focal mineralization	1	–	–	–	–	–	–	–
Capsular fibrosis	1	–	–	–	–	–	–	–
Stomach	4	4	4	4	–	–	–	–
Congestion	–	1	1	–	–	–	–	–
Sublingual gland	4	4	4	4	4	4	4	4
Lymphoid c. infiltr.	4	4	4	4	2	4	4	4
Focal mineralization	3	3	4	4	1	2	4	2
Submandibular gland	4	4	4	4	4	4	4	4
Lymphoid c. infiltr.	–	1	1	1	1	2	–	1
Testes	4	4	4	4	–	–	–	–
Tubular atrophy	–	–	1	–	–	–	–	–
Multinucleated cells	–	–	–	2	–	–	–	–
Thymus	4	4	4	4	4	4	4	4
Cyst(s)	2	4	3	3	2	–	2	1
Congestion	–	–	1	1	–	1	–	–
Thyroid gland	4	4	4	4	4	4	4	4
Cyst(s)	1	3	1	–	1	–	1	–
Lymphoid c. infiltr.	–	–	2	–	–	–	–	–
Focal mineralization	–	1	–	–	–	–	–	–
Tongue	–	–	–	–	4	4	4	4
Inflammatory c. focus	–	–	–	–	–	–	–	1
Urinary bladder	–	–	–	–	4	4	4	4
Congestion	–	–	–	–	2	3	–	1
Uterus	–	–	–	–	4	4	4	4
Diestrus	–	–	–	–	–	1	–	1
Cervicitis	–	–	–	–	1	–	–	–
Vagina	–	–	–	–	4	4	4	4
Inflammation	–	–	–	–	3	1	1	1

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