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Original Article

Spontaneous histopathology in New Zealand White rabbits: ten years of control data

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Abstract: The historical control database of a multinational laboratory services provider was queried for all histopathologic findings in New Zealand White rabbits which were used as control animals during a ten-year period (2011–2020). The query included all evaluated tissues, with or without microscopic findings, in studies conducted for safety testing for regulatory approval by the U.S. Food and Drug Agency (FDA) or the U.S. Environmental Protection Agency. A second query included studies conducted in the United Kingdom for control rabbits used in studies compliant with the Healthcare Products Regulatory Agency (MHRA) and/or the European Medicines Agency (EMA), which provide regulatory oversight in the United Kingdom and European Union, respectively. Infiltrates of inflammatory (mixed or mononuclear) cells were commonly noted in various organs including heart, digestive tract, muscle, thyroid, kidney, urinary bladder, eyelid, ocular structures, harderian gland, lacrimal gland, and lung. Mineralization was noted in aorta, kidney, urinary bladder, and ovary. Also noted were degeneration/necrosis in the myocardium, and intramuscular injection sites of the skin, degeneration/regeneration of muscle and diaphragm, ectopic tissue in the pancreas and thyroid, basophilic foci in salivary gland, increased/decreased vacuolation in adrenal gland, increased/decreased lymphocytic cellularity of lymph nodes, intrasinusoidal erythrocytes in lymph nodes, thymic atrophy, increased adipocytes in bone marrow, inflammatory cell foci in the liver and gall bladder, lacrimal gland atrophy, renal tubule basophilia, degeneration/regeneration, and dilatation; oviduct cyst; in the testis, degeneration/atrophy, cellular debris, dilatation, decreased sperm and segmental hypoplasia of seminiferous tubules; and squamous metaplasia of the testis and seminal vesicle. (DOI: 10.1293/tox.2023-0132; J Toxicol Pathol 2024; 37: 109–126)

Key words: background, histopathology, incidental, New Zealand White, microscopic, rabbit

Introduction

Drug developers and researchers involved in the safety testing of new chemical entities (new drug candidates, including vaccines or chemicals (pesticides, herbicides) to which people may be exposed), express interest in whether microscopic findings identified in safety/toxicity studies have been noted in control rabbits, and with what incidence. To our knowledge, the incidence of microscopic findings over a ten-year period in the historical control data base of a large, multinational, laboratory services provider has not been published. This article codifies the background and/or incidental findings noted in New Zealand White (NZW) rabbits aged, generally, from 12 to 17 weeks at initiation of study, to 46 weeks at euthanasia, in studies conducted in the United Kingdom (UK) or North America (NA), during the timeframe 2011–2020.

The purpose of this report, therefore, is to provide a reference for researchers, pathologists, and those concerned with drug and chemical safety studies in NZW rabbits, with occurrence and incidence data for spontaneously occurring microscopic findings unassociated with a test article. Tissues from the major organ systems examined in rabbits used in pre-clinical safety studies (cardiovascular, digestive, endocrine, hematolymphoid, hepatobiliary, integument, musculoskeletal, neural [including brain, spinal cord, and sciatic nerve], special senses [including eye and associated tissues],

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reproductive [female and male], respiratory and urinary systems) were addressed in this report. Findings occurring at greater than or equal to one percent incidence (i.e., number of affected animals per total number of evaluated animals in all studies queried) were considered relatively common. Microscopic findings of lower incidence were tabulated for information but may not have been discussed in the narrative. The most common or unexpected findings were addressed and compared with the literature. The highest incidence of findings in this study was noted in the hematolymphoid system of NZW rabbits; a high incidence of findings in the musculoskeletal system was likely due to the low number of rabbits in which these tissues were evaluated. Findings in the special senses (e.g., eye and the ocular adnexa), musculoskeletal, and/or reproductive tissues may generate particular interest, given the types of studies in which rabbits are used (e.g., developmental and reproductive), the nature and indications of new chemical entities tested in rabbits (e.g., dermal therapeutics and effects on bone), and tissue differences between rabbits and laboratory rodents (e.g., prostate and gastrointestinal system) as previously noted[1, 2].

Materials and Methods

The Labcorp historical control data base (HCDB) was queried of all NZW rabbit studies conducted between 01 January 2011 and 01 January 2021. Any duration of study was included, and all rabbits were aged, generally, 12 weeks to 17 weeks at the initiation of the study. The details of the United Kingdom (UK) studies from which these data were obtained are presented in Table 1. Data from the UK are based on samples from a total of 134 rabbits (6 from 3-week, 6 from 4-week, 32 from 6-week, 16 from 7-week, 8 from 11-week, 28 from 12-week, 12 from 13-week and 26 from 26-week studies) obtained from control groups of preclinical toxicological studies carried out between 2011 and 2019 at the Labcorp sites at Huntingdon and Eye. New Zealand White rabbits were obtained from accredited suppliers, with the majority (approximately 85%) obtained from Inotiv (formerly Envigo RMS) (Hillcrest, Loughborough, UK), and approximately 15% sourced from Highgate Farm, Market Rasen, Lincolnshire, UK, and Charles River, Saint-Germain-Nuelles, France. For the 3-week study, the control group was composed of 6 female NZW rabbits. For the 4-week study, the control groups were composed of 3 NZW rabbits per sex. For the 6-week studies, control groups comprised 5 to 8 NWZ rabbits per sex. For the 7-week study, control groups comprised 8 NWZ rabbits per sex. For the 11-week, control groups comprised 4 NWZ rabbits per sex. For the 12-week studies, control groups comprised 6 to 8 NWZ rabbits per sex. For the 13-week study, control groups were composed of 6 NWZ rabbits per sex, and for the 26-week studies, control groups comprised 4 to 9 NWZ rabbits per sex. In most studies, the age at the scheduled termination of the study was 14 to 28 weeks, except for approximately 19% of animals in the 26-week studies, where the age at termination was 42 to 46 weeks (Table 1 pertains).

Data from North America are based on tissues from studies conducted in Madison, WI (USA) and Greenfield, IN (USA) which were completed between 01 January 2011 and 01 January 2021. A total of 23 female-only studies and 21 male-only studies were conducted. NZW rabbits used in North America were obtained from accredited suppliers, Envigo (Denver, PA or Greenfield, IN, USA). Routes of administration were varied: one bolus intravenous (IV) study and one oral gavage study were 1-week long and involved only females. Two studies were 4 weeks long (one involved intramuscular injection and the other entailed topical instillation (presumably into the eye) of the test article; 4 studies (involving intramuscular injection, intravitreal ocular injection, or topical instillation [2 studies]) were 6 weeks long. Among the 11-week studies were 6 with intramuscular injection, and 1 each with intravitreal ocular injection or topical instillation. Three studies were 18 weeks long in which the route of administration was intra-articular injection, intramuscular injection, or intravitreal ocular injection. One study involved topical instillation for 26 weeks. Therefore, of the toxicity studies using rabbits in North America by the laboratory services provider currently known as Labcorp Early Development, 11 involved intramuscular injection, 5

Table 1. Details of Data Sources for New Zealand White Rabbits from Studies Conducted in the UK

Duration of study (weeks)	Total number of studies	Route of administration: Number of studies						Total number of control animals examined	
		Intramuscular injection	Intraocular injection	Subcutaneous injection	Rectal	Dermal topical	Intraperitoneal injection	Males	Females
3	1	-	-	-	-	-	1	0	6
4	1	-	-	-	-	1	-	3	3
6	3	2	-	-	1	-	-	19	13
7	1	-	-	1	-	-	-	8	8
11	1	1	-	-	-	-	-	4	4
12	2	2	-	-	-	-	-	14	14
13	1	-	1	-	-	-	-	6	6
26	2	1	-	1	-	-	-	13	13
Total	12 (F) 11 (M)	6	1	2	1	1	1	67	67

F: included females; M: included males.

used topical instillation, 3 used intravitreal ocular injection and 1 each involved bolus IV injection, intra-articular, or intra-spinal disc injection (Table 2 pertains).

The data presented here were obtained from control group animals in preclinical toxicity studies which were conducted in compliance with Good Laboratory Practices. Control animals were dosed with commonly used vehicles (e.g., sterile physiological saline, tris buffered saline, Ringer acetate) which are considered to have a negligible effect on the occurrence or incidence of background lesions[3]. All husbandry procedures, including light intensity and cage rotation, were similar between studies conducted in the US and UK; therefore, husbandry was not considered to have a differential effect on the incidence of microscopic findings in rabbits. Males were singly housed, and 2 to 3 females were housed in the same cage, in AAALAC International-accredited facilities in the UK. In the US, pregnant female rabbits were singly housed, to avoid altercations. Animal room temperature and humidity were automatically controlled at 15–16°C to 20–21°C and 40–45% to 70%, respectively, and filtered, non-recirculated, air was supplied. An automatic 12-hour light/dark cycle was maintained in most studies, with a 14-hour light/10-hour dark cycle used in one study. Animals had free access to potable water from the public supply in bottles with sipper tubes and were fed 150 g/animal of commercial pelleted rabbit diet in most studies, with one study restricting the daily food intake to 125 g/animal. In addition, a dietary supplement of whole meal/whole grain bread or hay was given daily, and, in some studies, 20 g of mixed raw vegetables was given twice weekly. Aspen Chew Blocks (soft white untreated wood blocks) and/or stainless-steel key rings were offered to all animals for environmental enrichment. The in-life experimental procedures undertaken during the UK studies were subject to the provisions of the United Kingdom Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (the Act), which conforms to the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, Council of Europe). In the U.S., all studies were performed in alignment with the Animal Welfare Act and its Regulations. A minimal number of animals were used, consistent with scientific integrity and regulatory acceptability, with consideration given to the welfare of individual animals in terms of the number and the extent of procedures to be conducted on each animal. All studies were reviewed and approved by the Institute Animal Care and Use Committee or Animal Welfare and Ethical Review Body of the respective site. Tissues were fixed in 10% neutral phosphate-buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE) for microscopic examination.

Results

The results included microscopic findings in twelve organ systems: cardiovascular, digestive, endocrine, hematolymphoid, hepatobiliary, integument, musculoskeletal, neural, special senses, reproductive [female and male], respiratory, and urinary systems. The Integument, Male and Female Reproductive systems and Special Senses were introduced with a brief background to facilitate understanding of the findings.

Cardiovascular system (Table 3)

Inflammatory cell infiltrates of the myocardium, in particular, mononuclear cell or mixed cell subtypes (the latter consisting of lymphocytes and/or plasma cells, macrophages, and roughly equal proportions of heterophils, eosinophils and/or basophils), were the most common background findings in male and female NZW rabbits (Fig. 1) and have been previously reported as common findings in the rabbit heart[1]. Increased cellularity (specifically lymphocyte or pigmented macrophage subtypes) was also reported for a few males, which may represent a historic term for inflammatory cell infiltrates. Other spontaneous findings, noted at a low incidence in one or both sexes, included: myocardial inflammation (Fig. 2); degeneration and/or degeneration/necrosis of the myocardium; fibrosis; hemorrhage; and vascular mineralization. A high incidence of cardiac inflammatory findings, occasionally in association with

Table 2. Details of Data Sources for New Zealand White Rabbits from Studies Conducted in North America

Duration of study (weeks)	Total number of studies	Route of administration: Number of studies							Total number of control animals examined	
		Bolus Intravenous injection	Intra-articular injection	Intra-muscular injection	Inter-vertebral disc injection	Intravitreal ocular injection	Oral gavage	Topical instillation (eye)	Males	Females
1	2	1 (females)	-	-	-	-	1 (females)	--	0	5
4	2	-	-	1	-	-	-	1	15	15
6	4	-	-	1	-	1	-	2	26	26
11	8	-	-	6	-	1	-	1	125	125
13	3	-	-	2	1	-	-	-	30	30
18	3	-	1	1	-	1	-	-	31	31
26	1	-	-	-	-	-	-	1	6	6
Total	23 (F) 21 (M)	1	1	11	1	3	1	5	233	238

F: included females; M: included males.

Table 3. Cardiovascular System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Heart				
Cellularity, increased, lymphocyte	1/273	0.4	0/210	0.0
Cellularity, increased, pigment macrophage	3/273	1.1	0/210	0.0
Degeneration, cardiomyocyte	3/273	1.1	0/210	0.0
Degeneration/necrosis	0/273	0.0	2/210	1.0
Fibrosis, myocardium	0/273	0.0	2/210	1.0
Hematocyst, valve	1/273	0.4	0/210	0.0
Hemorrhage	2/273	0.7	1/210	0.5
Inflammation, myocardium	0/273	0.0	1/210	0.5
Infiltrate, inflammatory cell, myocardium	0/273	0.0	4/210	1.9
Infiltrate, inflammatory cell, myocardium, mixed	23/273	8.4	24/210	11.4
Infiltrate, inflammatory cell, myocardium, mononuclear	30/273	11.0	32/210	15.2
Infiltrate, inflammatory cell, myocardium, NOS	0/273	0.0	3/210	1.4
Mineralization, vessel	2/273	0.7	3/210	1.4
Aorta				
Infiltrate, mononuclear cell	3/193	1.6	1/133	0.8
Intimal thickening	1/193	0.5	0/133	0.0
Mineralization	8/193	4.1	3/133	2.3

NOS: not otherwise specified.

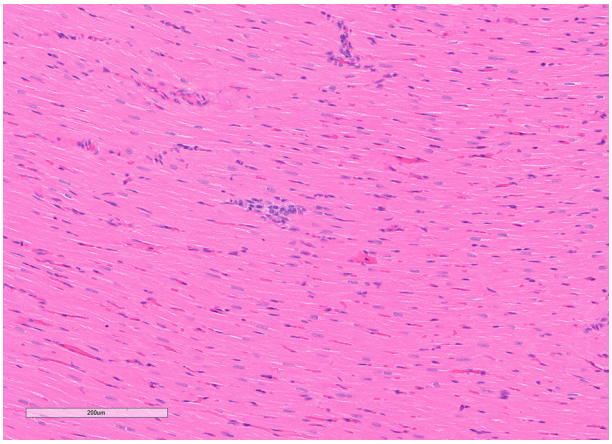

Fig. 1. Infiltrate, inflammatory cell, myocardium, heart. Bar=200 µm. H&E.

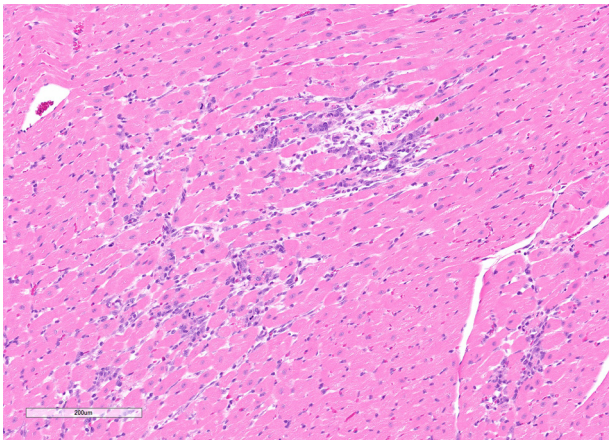

Fig. 2. Inflammation, myocardium, heart. Bar=200 µm. H&E.

low-grade cardiomyocyte necrosis and/or fibrosis, has been reported in association with the handling of female rabbits in non-clinical toxicity testing[4]. Myocardial adipocyte accumulation and cardiomyocyte mineralization are also reported as common background findings[1]; however, these findings were not reported for animals in our database.

In the aorta, mineralization was the most commonly reported finding in both sexes in our database as well as in the literature[1]. Mononuclear inflammatory cell infiltrates were occasionally noted in both sexes, and intimal thickening was recorded for one male; however, no hyperplastic or neoplastic findings were recorded in the cardiovascular system.

Digestive system (Table 4)

Cellular infiltrates, recorded as inflammatory cells, mononuclear or mixed cell infiltrates, were variably common findings throughout the digestive tract as reported previously[1].

Mixed cell inflammation of the duodenum, ileum or rectum, edema in the stomach, and acute inflammation in the rectum were observed at a lower incidence in males and/or females. In the esophagus, less common findings in males and/or females included muscle degeneration/regeneration, erosion/ulcer, and inflammation. There was a single incidence of tooth dysplasia noted in one male.

In the pancreas, ectopic splenic tissue was the most common finding while acinar cell necrosis and inflammatory cell infiltrates were recorded at a lower incidence.

In the parotid salivary gland, basophilic focus was

Table 4. Digestive System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Tongue				
Infiltrate, inflammatory cell, NOS	3/161	1.9	0/161	0.0
Tooth				
Dental dysplasia	1/1	100	0/0	0.0
Sublingual salivary gland				
Atrophy	0/55	0.0	1/103	1.0
Parotid salivary gland				
Atrophy, acinar cell	0/109	0.0	1/99	1.0
Atrophy, lobular	0/109	0.0	1/99	1.0
Atrophy, serous	1/109	0.9	0/99	0.0
Focus, basophilic	2/109	1.8	0/99	0.0
Inflammatory cell foci	0/109	0.0	1/99	1.0
Esophagus				
Degeneration/regeneration, muscle	2/186	1.1	0/187	0.0
Erosion/Ulcer	0/186	0.0	1/187	0.5
Infiltrate, inflammatory cell	0/186	0.0	0/187	0.0
Infiltrate, mononuclear cell	1/186	0.5	3/187	1.6
Inflammation	0/186	0.0	1/187	0.5
Stomach				
Infiltrate, mixed cell	1/170	0.6	0/170	0.0
Edema	0/170	0.0	1/170	0.6
Infiltrate, inflammatory cell	1/170	0.6	1/170	0.6
Duodenum				
Dilatation, gland	1/186	0.5	0/186	0.0
Infiltrate, inflammatory cell, mucosa/submucosa	4/186	2.2	1/186	0.5
Inflammation, mixed cell, mucosa	1/186	0.5	0/186	0.0
Jejunum				
Infiltrate, mixed cell	2/186	1.1	3/185	1.6
Ileum				
Inflammation, mixed cell	0/186	0.0	1/185	0.5
Infiltrate, inflammatory cell, submucosa	1/186	0.5	0/186	0.0
Infiltrate, mixed cell	1/186	0.5	2/186	1.1
Cecum				
Infiltrate, inflammatory cell, mucosa	0/185	0.0	1/183	0.5
Colon				
Infiltrate, inflammatory cell	1/186	0.5	0/186	0.0
Infiltrate, mononuclear cell	1/186	0.5	0/186	0.0
Rectum				
Inflammation, acute	1/145	0.7	0/144	0.0
Inflammation, mixed cell	0/145	0.0	1/144	0.7
Pancreas				
Ectopic tissue, splenic	2/185	1.1	5/184	2.7
Infiltrate, inflammatory cell, mononuclear	0/185	0.0	1/184	0.5
Necrosis, acinar cell	0/185	0.0	1/184	0.5

NOS: not otherwise specified.

commonly recorded; acinar cell atrophy, inflammatory cell foci, and lobular atrophy were noted at 1% incidence.

Other findings recorded for tissues in the digestive system were uncommon in our databases.

Endocrine system (Table 5)

In New Zealand White rabbits, the most common findings in the adrenal cortex consisted of increased or decreased vacuolation, followed by congestion/hemorrhage, angiectasis, and extracapsular cortical tissue. Other less common findings were hypertrophy of adrenocortical cells, cysts, and mononuclear cell infiltrates, each noted in a single male.

In the thyroid, inflammatory cell infiltrate, not otherwise specified (NOS) was the most common finding followed by presence of ectopic thymus and follicular dilatation. Other uncommon findings included C-cell hyperplasia, ectopic salivary gland, and C-cell adenoma.

Findings in the parathyroid gland and pituitary consisted of cysts and were uncommon in our database.

Hematolymphoid system (Table 6)

In the hematolymphoid system of NZW rabbits, microscopic findings were reported in eleven different lymph

Table 5. Endocrine System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Males		Females	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Adrenal cortex				
Angiectasis	0/209	0.0	1/208	0.5
Congestion/hemorrhage	3/209	1.4	0/208	0.0
Cyst	1/209	0.5	0/208	0.0
Ectopic tissue, adrenocortical	1/209	0.5	1/208	0.5
Hypertrophy	1/209	0.5	0/208	0.0
Infiltrate, mononuclear cell	1/209	0.5	0/208	0.0
Pigment, macrophage	1/209	0.5	0/208	0.0
Vacuolation, decreased	2/153	1.3	0/208	0.0
Vacuolation, increased	2/209	1.0	1/208	0.5
Parathyroid				
Cyst	0/163	0.0	1/157	0.6
Pituitary				
Cyst	1/185	0.5	1/179	0.6
Thyroid				
Adenoma, C-cell	1/187	0.5	0/186	0.0
Cyst	1/187	0.5	0/186	0.0
Ectopic tissue, salivary gland	0/187	0.0	1/186	0.5
Ectopic tissue, thymus	7/187	3.7	13/186	7.0
Follicular dilatation	3/187	1.6	1/186	0.5
Hyperplasia, c-cell	1/187	0.5	0/186	0.0
Infiltrate, inflammatory cell, NOS	34/187	18.1	36/186	19.3

NOS: not otherwise specified.

nodes and most often consisted of changes in the cellularity of lymphocytes (increased or decreased) or macrophages (increased with or without pigment or vacuolation), the presence of erythrocytes in sinusoids (Fig. 3) with or without erythrophagocytosis, and pigment. Infiltrate, inflammatory cells (heterophils) was occasionally noted (Fig. 4).

In the marrow of the femur and sternum, the most reported microscopic finding was increased adipocytes. Increases in hematopoietic cells and the presence of fibrosis in the femoral marrow were noted in a few studies. The marrow of the rib was examined in eleven studies without the detection of abnormalities. In the spleen, increased perivascular inflammatory cells were commonly reported in males. The most frequently reported findings in the thymus included atrophy/involution and decreased cellularity of lymphocytes.

Hepatobiliary system (Table 7)

In the liver, inflammatory cell foci were the most common finding followed by inflammatory cell infiltrates in the portal areas and rarefaction of hepatocellular cytoplasm. Other findings of lower incidence included mixed cell inflammation, subcapsular cyst, portal inflammation, and hepatocellular cytoplasmic vacuolation. A single incidence of hepatic lobar necrosis was noted in this review. Findings in the gall bladder consisted of inflammatory cell infiltrates reported in one male and mononuclear cell infiltrates in one female.

Integumentary system

Rabbit skin has been used over decades for the safety assessment of many compounds, such as pharmaceuticals, vaccines, and chemicals. Modes of administration include topical or injected (including subcutaneous, intramuscular, intravenous). The elongated pinnae are most often used for the intravenous administration of test substances as well as blood sampling. The epidermis of both the pinnae and body is thin in rabbits[5]. Different follicle types, generally arranged in clusters, produce the three different hair types in rabbit fur: guard hairs, awn hairs, and down hairs[1]. Rabbit hair follicles have large numbers of associated dermal sebaceous glands, but sweat glands are generally either absent or vestigial[5]. Several specialized glands are found in the skin from various anatomic regions and are generally involved with specific behaviors[1].

The skin, including the subcutis, is generally sampled from the ventral abdomen, inguinal region, and examined as a single tissue on general toxicity studies; however, if dermal or other sites of test article administration are present, these are generally identified and microscopically evaluated separately from a corresponding area of untreated skin/subcutis. On this basis, the section covering the integument has been subdivided accordingly.

Skin (Table 8)

Inflammation (NOS) and/or inflammatory cell infiltrates were the most commonly reported background findings in the skin (from any location) within the database. Uncommon findings included granulomatous and pyogranulomatous inflammation, erosion/ulcer, degeneration, and hemorrhage. In one instance, treated skin was specified as a tissue, but with no abnormalities detected.

Inflammatory lesions (inflammation, mononuclear cell infiltrates), epidermal exudate, and fibrosis/fibroplasia within the skin over intramuscular injection site sections were

Table 6. Hematolymphoid System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Males		Females	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Lymph node, axillary				
Cellularity increased, macrophage	2/62	3.2	0/62	0.0
Erythrocytes, intrasinusoidal	8/62	12.9	8/49	16.3
Lymph node, cervical				
Erythrocytes, intrasinusoidal	1/28	3.6	0/30	0.0
Lymph node, iliac				
Congestion	1/9	11.1	0/10	0.0
Pigment	0/9	0.0	1/10	10.0
Lymph node, inguinal				
Atrophy	1/94	1.1	0/93	0.0
Cellularity increased, macrophage	6/114	5.3	0/93	0.0
Infiltrate, macrophage, vacuolated	0/94	0.0	2/93	2.2
Lymph node, lumbar				
Cellularity increased, macrophage	2/37	5.4	0/36	0.0
Erythrocytes, intrasinusoidal	15/37	40.5	11/36	30.6
Lymph node, mandibular				
Cellularity decreased, lymphocyte	3/270	1.1	1/270	0.4
Cellularity increased, lymphocyte, paracortex	3/313	1.0	1/313	0.3
Congestion	0/270	0.0	1/270	0.4
Erythrocytes, intrasinusoidal	7/83	8.4	6/313	1.9
Erythrophagocytosis	2/270	0.7	0/270	0.0
Lymphangiectasis	0/270	0.0	1/270	0.4
Lymph node, mediastinal				
Erythrocytes, intrasinusoidal	1/1	100.0	0/9	0.0
Pigment macrophages increased	1/1	100.0	0/9	0.0
Lymph node, mesenteric				
Cellularity increased, follicles	0/46	0.0	1/49	2.0
Cellularity increased, lymphocyte	6/197	3.0	4/201	2.0
Cellularity increased, lymphocyte (germinal center)	1/46	2.2	3/49	6.1
Cellularity increased, pigmented macrophage	6/243	2.5	0/250	0.0
Erythrocytes, intrasinusoidal	4/243	1.6	5/201	2.5
Lymph node, parotid				
Cellularity decreased, lymphocyte	2/80	2.5	1/80	1.3
Lymph node, prescapular				
Infiltrate, macrophage, vacuolated	1/4	25.0	1/1	100.0
Lymph node, cervical				
Erythrocytes, intrasinusoidal	1/28	3.6	1/1	100.0
Marrow, femur				
Cellularity increased, adipocytes	20/128	15.6	19/128	14.8
Cellularity, increased, hematopoietic cells	0/128	0.0	1/128	0.8
Fibrosis, marrow	1/44	2.3	0/38	0.0
Marrow, sternum				
Cellularity increased, adipocytes	20/145	13.8	18/144	12.5
Spleen				
Infiltrate, inflammatory cell, perivascular	1/68	1.5	0/68	0.00
Thymus				
Atrophy/involution	16/263	6.1	29/247	11.7
Cellularity decreased, lymphocyte	9/208	4.3	8/200	4.0
Cellularity increased, macrophage	4/263	1.5	0/247	0.0
Ectopic tissue, parathyroid	1/208	0.5	2/200	1.0
Infiltrate, macrophage	0/208	0.0	1/200	0.5
Inflammation, mixed cell	0/208	0.0	1/200	0.5

also infrequently reported.

Skin lesions which have been reported in the literature are primarily inflammatory and include pododermatitis, ulcerative dermatitis[6], moist dermatitis, and alopecia associated with fighting in group-housed males[1]. Bradley *et al.*[1] reported epidermal edema, fibrosis, mineralization, necrosis, pigment, and thrombosis as common findings in New Zealand White rabbit skin; however, none of these findings were recorded in our database.

Intramuscular injection site (Table 9)

Overall, inflammation and degeneration and/or necrosis were the most frequently reported findings for intramuscular injection sites. These data were difficult to interpret

as a variety of specific modifiers were used to describe the inflammation including subacute, chronic, chronic-active, mixed cell, mononuclear cell, and NOS; the use of other modifiers (including the specific location within the section) also varied between findings. The descriptive terminology used to qualify the finding of degeneration/necrosis varied

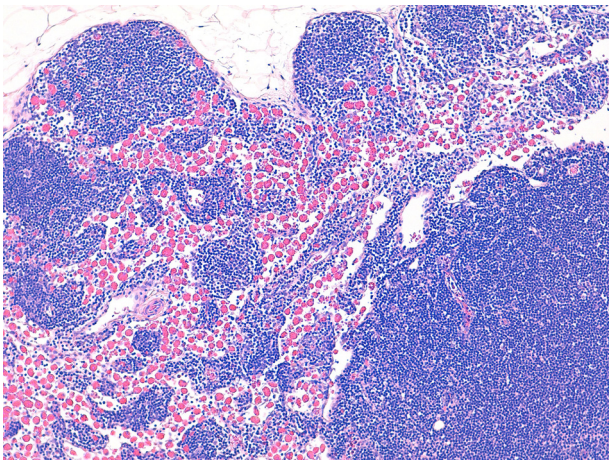

Fig. 3. Lymph node, parotid, erythrocytes, intrasinusoidal. 10×, H&E.

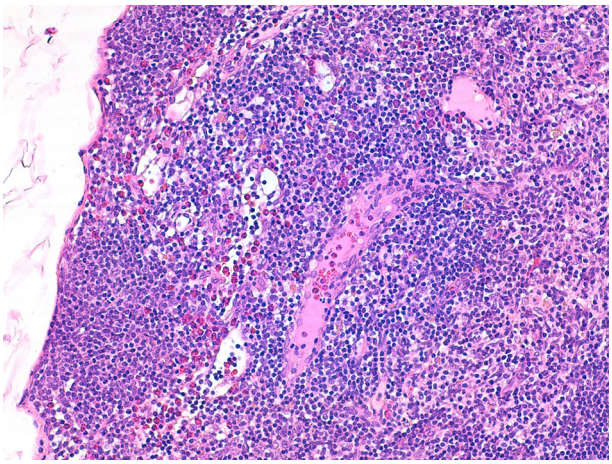

Fig. 4. Lymph node, tracheobronchial, infiltrate, inflammatory cells (mostly heterophils). 20×, H&E.

Table 7. Hepatobiliary System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Males		Females	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Liver				
Cyst, subcapsular	0/219	0.0	1/220	0.5
Foci, inflammatory cells	11/219	5.0	15/220	6.8
Infiltrate, inflammatory cell, NOS	1/219	0.5	0/220	0.0
Infiltrate, inflammatory cell, portal	3/219	1.4	11/220	5.0
Inflammation, mixed cell	0/219	0.0	1/220	0.5
Inflammation, portal	0/219	0.0	2/220	0.9
Necrosis, lobe	0/219	0.0	1/220	0.5
Rarefaction, cytoplasm	10/219	4.6	5/220	2.3
Vacuolation, cytoplasm	1/219	0.5	0/220	0.0
Vacuolation, cytoplasm centrilobular	0/219	0.0	1/220	0.5
Gall bladder				
Infiltrate, inflammatory cell	1/171	0.6	0/171	0.0
Infiltrate, mononuclear cell	0/171	0.0	1/171	0.6

NOS: not otherwise specified.

Table 8. Integumentary System—Incidence of Microscopic Findings in the Skin of New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Skin[a]				
Degeneration	0/261	0.0	1/255	0.4
Erosion/ulcer	0/204	0.0	1/255	0.4
Hemorrhage	0/261	0.0	1/255	0.4
Inflammation, granulomatous	0/261	0.0	1/255	0.4
Inflammation, NOS	7/261	2.7	8/255	3.1
Inflammation, pyogranulomatous	1/261	0.4	1/255	0.4
Skin, anus/perianal				
Infiltrate, inflammatory cell, mixed	2/6	33.3	0/0	0.0

[a]: routine/control section, ventral abdomen, inguinal region (30–50 mm diameter, retained on cardboard). NOS: not otherwise specified.

and included: degeneration, necrosis, myofiber degeneration/regeneration, degeneration/regeneration in the quadriceps muscle, and also myofiber basophilia/regeneration. Other reported non-specific background lesions in the intramuscular injection site included foreign material/body, needle-track lesions, and hemorrhage.

Intravenous injection site (Table 10)

The intravenous injection site was much less frequently examined in comparison to intramuscular injection sites in our database, therefore findings in this site were generally recorded at a higher apparent incidence due to the reduced number of animals examined. Perivascular inflammation (mixed cell) was recorded in one male and one female; thrombus and hemorrhage were each recorded once in females.

Musculoskeletal system (Table 11)

Seventy-seven studies included the examination of specific muscles, including the biceps brachii, diaphragm, gastrocnemius, ocular, psoas, quadriceps, and a general category, skeletal muscle. Microscopic findings noted in named muscles were limited to inflammation or inflammatory cell infiltrate, which were noted in the diaphragm, quadriceps and, to a lesser extent, the biceps brachii muscle. Infiltrates of mononuclear leukocytes were reported in the skeletal muscle associated with the stifle joint. The second most common microscopic finding in skeletal muscle was degeneration of myofibers (with or without regeneration) associated with the stifle joint and, to a much lesser extent, of the diaphragm. One of 49 females evaluated (2.0%) was noted with myofiber degeneration in skeletal muscle.

No abnormalities were noted microscopically in the bone of the femur, rib (with or without costochondral junction), or sternum.

Nervous system (Table 12)

The brain only had uncommonly reported background findings, which included axonal degeneration in the optic tract/chiasm, gliosis, and inflammatory cell infiltrate.

Gliosis and inflammatory cell infiltrate have been reported to be uncommon findings in the rabbit brain[1]. No microscopic abnormalities were reported in the spinal cord and sciatic nerve in our database.

Table 9. Integumentary System—Incidence of Microscopic Findings in the Intramuscular Injection Site of New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Injection site, intramuscular				
Basophilia/regeneration, myofiber	2/287	0.7	5/234	2.1
Degeneration	1/287	0.3	2/234	0.9
Degeneration/necrosis, myofiber, skeletal muscle	17/287	5.9	25/234	10.7
Degeneration/regeneration, quadriceps	2/287	0.7	0/234	0.0
Degeneration/regeneration, subcutaneous	1/287	0.3	0/234	0.0
Exudate, epidermal surface	2/287	0.7	1/234	0.4
Fibrosis/fibroplasia, dermis	1/287	0.3	1/234	0.4
Foreign material/foreign body (hair shaft)	2/287	0.7	3/234	1.3
Hemorrhage	8/287	2.8	3/234	1.3
Infiltrate, mononuclear cell	2/287	0.7	1/234	0.4
Infiltrate, mononuclear cell, dermis	1/287	0.3	0/234	0.0
Inflammation	21/287	7.3	28/234	12.0
Inflammation, chronic	9/287	3.1	9/234	3.8
Inflammation, dermis, chronic	1/287	0.3	0/234	0.0
Inflammation, skin	1/287	0.3	1/234	0.4
Inflammation, chronic-active	1/287	0.3	5/234	2.1
Inflammation, dermis/subcutis, chronic-active	1/287	0.3	2/234	0.9
Inflammation, mixed cell	12/287	4.2	3/234	1.3
Inflammation, subacute	2/287	0.7	3/234	1.3
Necrosis	3/287	1.0	5/234	2.1
Needle-track lesion	0/287	0.0	3/234	1.3

Table 10. Integumentary System—Incidence of Microscopic Findings in the Intravenous Injection Site of New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Injection site, intravenous				
Inflammation, mixed cell, perivascular	1/5	20.0	1/5	20.0
Hemorrhage	0/5	0.0	1/5	20.0
Thrombus	0/5	0.0	1/5	20.0

Table 11. Musculoskeletal System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Males		Females	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Joint, stifle				
Degeneration, myofiber	3/18	16.7	0/6	0.0
Degeneration, myofiber, skeletal muscle	1/18	5.6	0/6	0.0
Infiltrate, mononuclear cells, skeletal muscle	5/18	27.8	0/6	0.0
Muscle, biceps brachii				
Degeneration, myofiber	0/77	0.0	1/77	1.3
Inflammation	1/29	3.4	1/29	3.4
Inflammatory cell foci	0/29	0.0	1/29	3.4
Diaphragm				
Degeneration/regeneration	1/30	3.3	0/28	0.0
Inflammation	3/14	21.4	4/15	26.7
Muscle, quadriceps, left				
Infiltrate, inflammatory cells	2/10	20.0	0/10	0.0
Inflammation, acute	0/10	0.0	1/10	10.0
Muscle, skeletal				
Degeneration, myofiber	0/55	0.0	1/49	2.0

Table 12. Nervous System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence rate (%)	Occurrence/ No. examined	Incidence rate (%)
Brain				
Degeneration, axon, optic tract/chiasm	0/203	0.0	1/197	0.5
Gliosis	1/203	0.5	1/197	0.5
Infiltrate, inflammatory cell	1/203	0.5	1/197	0.5

Reproductive Tissues

Female reproductive system (Table 13)

Female rabbits become sexually mature at approximately five months of age, therefore, based on age of study start and at necropsy, rabbits used for routine nonclinical studies are generally peripubertal to fully mature, as noted in Table 13 where maturity was recorded. Rabbits are induced ovulators, hence decreased or absent corpora lutea, as well as occasional corpora hemorrhagica, are normal background findings in the ovary. Mineralization (Fig. 5) is described by Bradley *et al.*[1] as an uncommon finding; however, it was recorded with the highest incidence in studies in our database. Other background findings included: interstitial cell vacuolation and follicular cysts; the latter are considered a common background finding[1]. Atrophy (age-related or otherwise) is not reported in our database but is also considered to be a common background finding[1].

By six months of age, rabbit ovaries have prominent interstitial tissue composed of large, lipid-dense, polygonal cells which primarily produce progesterone due to high 3, β-hydroxysteroid dehydrogenase activity[7]. In addition, villous processes are common on the surface epithelium (mesothelium) of the rabbit ovary (Fig. 6) but are not reported on adjacent reproductive tract structures[8]. These two findings may have been recorded as prominent interstitial cells and mesothelial cell hyperplasia, respectively, in the database. Aside from mesothelial cell hyperplasia, no other hyperplastic or neoplastic findings were recorded for the female NZW rabbits included in the database, likely due to the young age of these animals on nonclinical studies.

Cysts were recorded in the database for the oviduct

Table 13. Female Reproductive System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Occurrence/ No. examined	Incidence (%)
Ovary		
Corpora lutea, absent	4/194	2.1
Cyst, follicular	1/194	0.5
Hemorrhage, follicle	1/194	0.5
Hyperplasia, mesothelial cell	1/194	0.5
Mature	42/194	21.6
Mineralization	17/194	8.8
Prominent interstitial cells	1/194	0.5
Vacuolation, interstitial cell	2/194	1.0
Oviduct		
Cyst	5/106	4.7
Uterus		
Dilatation, luminal	1/186	0.5
Mineralization	1/186	0.5
Vagina		
Mineralization	2/186	1.1
Mammary Gland		
Dilatation	1/171	0.6
Mineralization	1/171	0.6
Secretions, increased	4/171	2.3

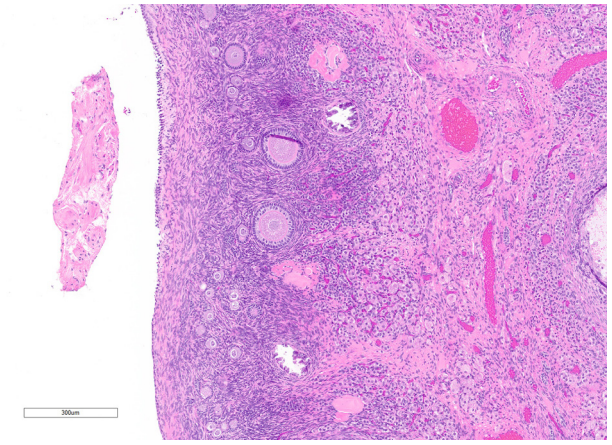

Fig. 5. Mineralization, ovary. Bar=300 µm. H&E.

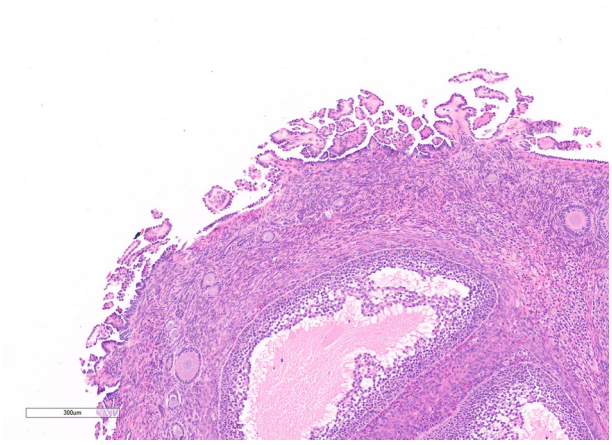

Fig. 6. Mesothelium (normal), ovary. Bar=300 µm. H&E.

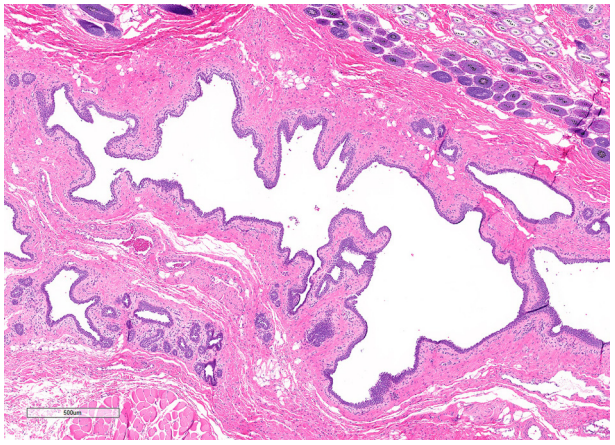

Fig. 7. Dilatation, mammary gland. Bar=500 µm. H&E.

from five females, but not recorded in the uterus, cervix, or vagina, although this finding is considered to be a common background finding in all of these structures[1].

Other findings recorded for the uterus and vagina included luminal dilatation and mineralization; these are considered uncommon background findings by Bradley *et al.* No findings were recorded for the clitoral gland for animals in the database.

Mammary glandular dilatation (Fig. 7), increased secretions, and mineralization were recorded for a few animals. Mineralization is reported to be a common background finding in female rabbits; other common background findings not noted in the database included: fibrosis, hemorrhage, epithelial hypertrophy, inflammatory cell infiltrates, and lobuloalveolar hyperplasia[1].

Male reproductive system (Table 14)

Male rabbits are considered sexually mature by six months of age, based on the onset of mounting behavior, the production of spermatozoa in semen, and the physical and chemical characteristics of the ejaculate[9]. The testis and epididymis of NZW rabbits are anatomically similar to those of other animals used in non-clinical safety testing[10]. Based on the age at study start (12–17 weeks) and the duration of the study (1–26 weeks), the majority of males in our database were likely peripubertal or mature; sexually maturity was not regularly recorded. Tubular atrophy (Fig. 8) and degeneration/atrophy, tubular dilation, and multinucleated giant cells (Fig. 9) were recorded with the highest incidence. Tubular atrophy and multinucleated giant cells are considered common spontaneous background findings, along with Ley-

Table 14. Male Reproductive System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Occurrence/ No. examined	Incidence (%)
Testis		
Degeneration/atrophy, tubule	20/206	9.7
Dilatation, rete testis	1/206	0.5
Dilatation, tubule	9/206	4.4
Hyperplasia, mesothelial cells	1/206	0.5
Hypoplasia, segmental	3/206	1.5
Immature	3/206	1.5
Mature	33/206	16.0
Multinucleated giant cells	8/206	3.9
Peripubertal	8/206	3.9
Vacuolation, tubule	1/206	0.5
Epididymis		
Aspermia	2/198	1.0
Cell debris, lumen	21/198	10.6
Infiltrate, mononuclear cell	1/198	0.5
Spermatocele	1/198	0.5
Sperm decreased, lumen	4/198	2.0
Vacuolation, epithelium	1/198	0.5
Prostate		
Edema	1/198	0.5
Hyperplasia, epithelium	1/198	0.5
Infiltrate, inflammatory cell	3/198	1.5
Inflammation, neutrophilic	1/198	0.5
Metaplasia, squamous	18/198	9.1
Seminal vesicle		
Hemorrhage	1/197	0.5
Metaplasia, squamous	4/197	2.0

dig cell vacuolation and Leydig cell atrophy[1].

Other findings recorded at a low incidence included: segmental hypoplasia; mesothelial cell hyperplasia; and dilation of the rete testis. Tumors were not recorded in the male reproductive tissues for any male rabbits in the database, which likely reflects the young age of male rabbits used in nonclinical toxicity testing.

In the epididymis, luminal cell debris (Fig. 10) was the most frequently recorded finding in NZW rabbits. Uncommon findings included: decreased luminal sperm, aspermia, epithelial vacuolation, and a single spermatocele.

The male accessory sex glands in the rabbit are anatomically different to those from other species used in nonclinical toxicity resting. The rabbit vas deferens opens into large, bilobed ampullae, which lie dorsal to the urethra. The seminal vesicles (vesicular glands) appear as a single sac-like structure and lie between the prostate complex dorsally and the urinary bladder ventrally. The prostate is composed of a cranial proprostate, the prostate, and two lateral lobes of paraprostate which are histologically identical to the prostate. The bulbourethral gland is bilobed and lies caudally to the prostate complex, dorsal to the urethra[1, 2].

Squamous metaplasia was the most frequently recorded finding in the prostate (Fig. 11) and seminal vesicles and is considered a common background finding in the prostate[1, 11]. Other findings that were recorded at a low incidence include: inflammatory cell infiltrates, neutrophilic inflammation, epithelial hyperplasia, and edema in the prostate, and hemorrhage in the seminal vesicle. No microscopic observations were recorded for the efferent ducts, vas deferens, ampullae, bulbourethral glands, preputial (inguinal) glands, or mammary glands.

Respiratory system (Table 15)

Inflammatory changes and cellular infiltrates (Fig. 12) were the most common background changes observed in the lungs of control NWZ rabbits, followed by hemorrhage. All other changes were uncommon. Within the inflammatory changes, infiltrate of inflammatory cells, NOS was the most common change observed, followed by the mixed cell type,

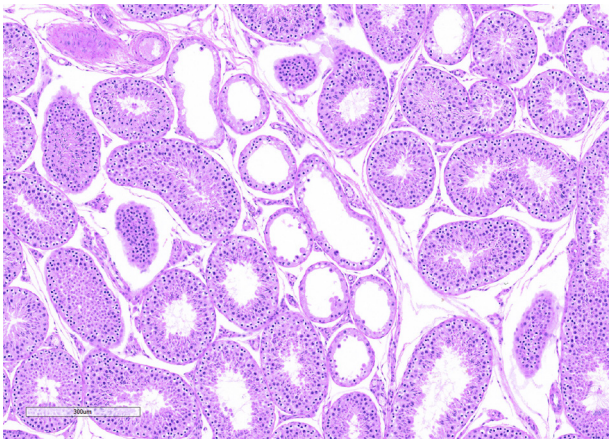

Fig. 8. Atrophy, tubular, testis. 10×. H&E.

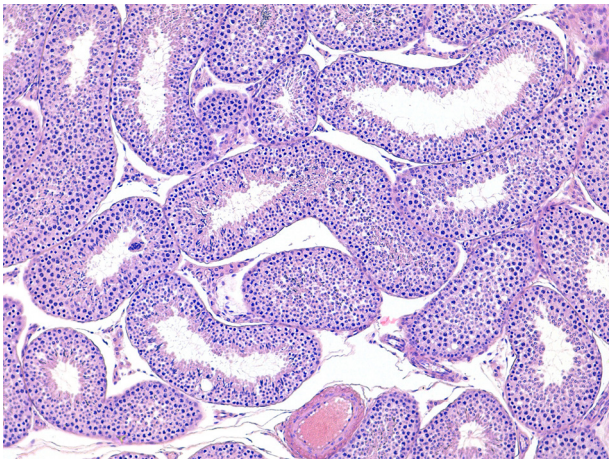

Fig. 9. Multinucleated giant cell, testis. 10×. H&E.

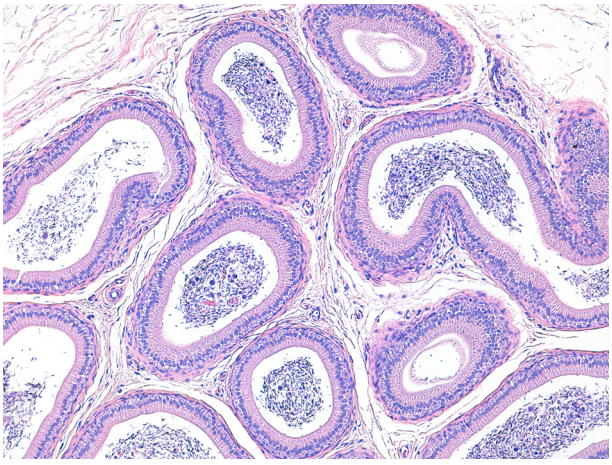

Fig. 10. Cell debris, lumen, epididymis. 10×. H&E.

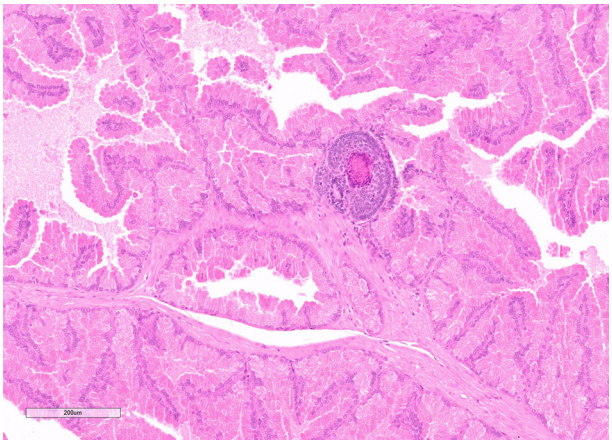

Fig. 11. Metaplasia, squamous cell, prostate. Bar=200 µm. H&E.

(Fig. 13), both of which were seen at similar incidences in both sexes. Inflammation (granulomatous, chronic, and mixed cell) and edema were observed at a low incidence. Hemorrhage was seen at a slightly higher incidence in males than in females, and all other changes such as alveolar macrophage aggregations, osseous metaplasia, mineralization, and thrombus were observed at a low incidence. Background pathology in the trachea was rare, with epithelial vacuolation, eosinophilic globules, and inflammatory cell infiltrates observed at a low incidence.

Special Senses

Among special senses systems, olfactory and otic systems are rarely evaluated in general toxicity studies in rabbits, leading to a paucity of historical control data from these two systems; however, due to their smaller size and ease of handling, rabbits are frequently used in experimental ophthalmology and visual science studies including ocular toxicity studies. Moreover, the presence of a large globe for a small animal species makes them a more relevant second species than rodents in Investigational New Drug (IND)-enabling ocular programs. In this section, historical control data is presented for eye and ocular adnexa (glands and other eye-associated tissues excluding eye and optic nerve).

Eye and the ocular adnexa (Table 16)

In the eyelid, inflammation (NOS) and mononuclear cell infiltrate were the commonly reported background findings in both male and female NZW rabbits. Mixed cell infiltrate and chronic inflammation were common in the eyelid but were limited to females. These findings were reported

Table 15. Respiratory System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Lung				
Edema	1/197	0.5	0/193	0.0
Hemorrhage	4/197	2.0	1/193	0.5
Infiltrate, inflammatory cell, NOS	18/197	9.1	21/193	10.9
Inflammation, alveoli	0/197	0.0	3/193	1.6
Inflammation, chronic	1/197	0.5	0/193	0.0
Inflammation, granulomatous	2/197	1.0	0/193	0.0
Inflammation, mixed cell	3/197	1.5	5/193	2.6
Macrophage increased, alveolar	1/197	0.5	1/193	0.5
Metaplasia, osseous	1/197	0.5	1/193	0.5
Mineralisation, NOS	0/197	0.0	1/193	0.5
Mineralisation, alveolar septa	0/197	0.0	1/193	0.5
Thrombus	0/197	0.0	1/193	0.5
Trachea				
Eosinophilic globules	1/186	0.5	1/180	0.6
Infiltrate, inflammatory cell, lamina propria	0/186	0.0	1/180	0.6
Vacuolation, epithelium	1/186	0.5	1/180	0.6

NOS: not otherwise specified.

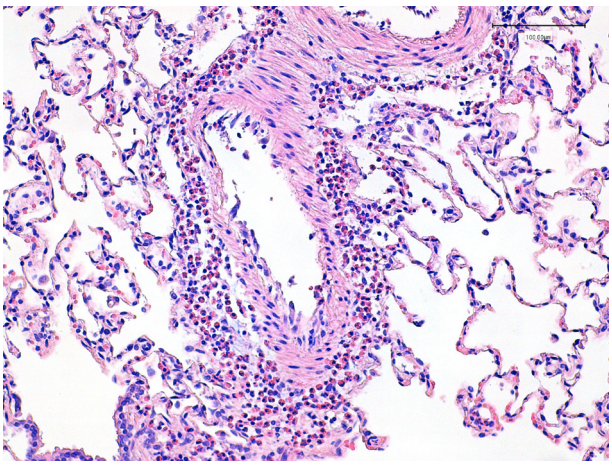

Fig. 12. Infiltrate, inflammatory cell, perivascular. 20×, H&E.

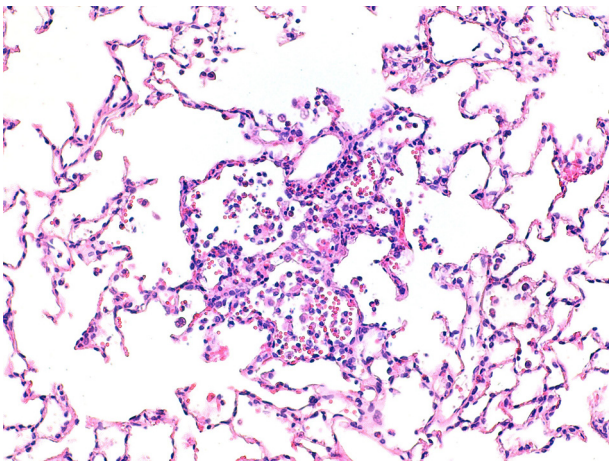

Fig. 13. Inflammatory mixed cell infiltrate, alveoli. 20×, H&E.

Table 16. Eye and the Ocular Adnexa—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
EYE				
Bulbar conjunctiva				
Granuloma, foreign body	0/188	0.0	1/212	0.5
Infiltrate, granulocytes	0/188	0.0	2/212	0.9
Infiltrate, mixed cell	2/188	1.1	0/212	0.0
Infiltrate, mononuclear cell	3/188	1.6	9/212	4.2
Inflammation, NOS	5/188	2.7	3/212	1.4
Choroid				
Infiltrate, mononuclear cell	1/344	0.3	3/313	1.0
Pigment	0/344	0.0	1/313	0.3
Ciliary body				
Edema	0/344	0.0	1/313	0.3
Vascular anomaly	1/344	0.3	0/313	0.0
Cornea				
Infiltrate, mononuclear cell	1/344	0.3	1/313	0.3
Lens				
Degeneration	14/344	4.1	10/313	3.2
Retina				
Atrophy	0/344	0.0	1/313	0.3
Degeneration	4/344	1.2	8/313	2.6
Developmental anomaly/disorganization	1/344	0.3	1/313	0.3
Fold	1/344	0.3	0/313	0.0
Hypertrophy, retinal pigment epithelium (RPE)	1/344	0.3	1/313	0.3
Infiltrate, mononuclear cell	1/344	0.3	0/313	0.0
Rosette	0/344	0.0	2/313	0.6
Vitreous				
Infiltrate, mixed cell	0/344	0.0	1/313	0.3
OCULAR ADNEXA				
Eyelid				
Infiltrate, mixed cell	1/174	0.6	4/198	2.0
Infiltrate, mononuclear cell	7/174	4.0	5/198	2.5
Inflammation, chronic	0/174	0.0	5/198	2.5
Inflammation, NOS	11/174	6.3	22/198	11.1
Harderian gland				
Atrophy	17/285	6.0	5/304	1.6
Infiltrate, eosinophils	1/285	0.4	1/304	0.3
Infiltrate, mixed cell	0/285	0.0	1/304	0.3
Infiltrate, mononuclear cell	15/285	5.3	15/304	4.9
Inflammation, NOS	10/285	3.5	7/304	2.3
Lacrimal gland alteration	3/285	1.1	6/304	2.0
Lacrimal gland				
Atrophy	0/262	0.0	2/290	0.7
Infiltrate, mononuclear cell	3/262	1.1	7/290	2.4
Nictitating membrane				
Inflammation, chronic	1/42	2.4	1/42	2.4
Palpebral conjunctiva				
Dilation, Meibomian gland	1/174	0.6	3/198	1.5
Hyperplasia, lymphoid	4/174	2.3	5/198	2.5
Infiltrate, granulocytes	16/174	9.2	16/198	8.1
Infiltrate, mononuclear cell	19/174	10.9	17/198	8.6
Inflammation, granulomatous, Meibomian gland	1/174	0.6	0/198	0.0
Inflammation, NOS	1/174	0.6	0/198	0.0
Retina				
Atrophy	0/344	0.0	1/313	0.3
Degeneration	4/344	1.2	8/313	2.6
Developmental anomaly/disorganization	1/344	0.3	1/313	0.3
Fold	1/344	0.3	0/313	0.0
Hypertrophy, retinal pigment epithelium (RPE)	1/344	0.3	1/313	0.3
Infiltrate, mononuclear cell	1/344	0.3	0/313	0.0
Rosette	0/344	0.0	2/313	0.6

NOS: not otherwise specified.

to occur uncommonly previously[1]. However, mononuclear infiltrate was also reported to occur commonly in another study[12].

Inflammation (NOS) and mononuclear cell infiltrate were commonly reported background findings in the bulbar conjunctiva in eyes of both sexes. All other changes, such as mixed cell infiltrate (1.1% in males), granulocyte infiltrate, and foreign body granuloma, were observed at a low incidence.

In the palpebral conjunctiva, mononuclear cell infiltrate, granulocyte infiltrate and lymphoid hyperplasia were common background findings in both sexes, whereas Meibomian gland dilatation was reported as a common background finding only in females. Other findings present at a lower incidence in the palpebral conjunctiva were granulomatous inflammation in the Meibomian gland and inflammation (NOS). Mononuclear cell infiltrate was uncommon reported in the cornea of both sexes.

Mononuclear cell infiltrate was reported as a common finding in the female choroid; uncommon findings, present at lower incidence, were pigment in the choroid, and vascular anomaly or edema in the ciliary body.

In the lens, in contrast to the previous report[1], degeneration was a common background finding that occurred at an incidence of 4.1% and 3.2% in males and females, respectively. Mixed cell infiltrate in the vitreous fluid was uncommon in both sexes.

In the retina, the only common background finding that occurred in both sexes was degeneration; all other findings including fold, rosette, developmental anomaly/disorganization, atrophy, hypertrophy of retinal pigmented epithelium, and mononuclear cell infiltrate were uncommonly reported both sexes. Retinal degeneration was not commonly reported in the rabbit previously[1].

No microscopic background findings were reported in the optic nerve.

In the Harderian gland, atrophy, mononuclear cell infiltrate, inflammation (NOS) and lacrimal gland alteration were commonly reported in our data base, and in a previously published study[12]. These findings, however, have been reported at lower incidences[1]. Eosinophil infiltrate and mixed cell infiltrate were uncommon findings Harderian gland.

In the lacrimal gland, mononuclear cell infiltrate was commonly reported in both sexes; atrophy was uncommon and only reported in females. Chronic inflammation was common in the nictitating membrane of both sexes.

Urinary system (Table 17)

Tubular basophilia was the most common background change observed in the kidneys of NZW rabbits, followed by mineral deposits, inflammatory changes, tubular dilatation, degeneration and/or regeneration, casts, cysts, cortical fibrosis, and pigment, with other changes such as hemorrhage, thrombus, calculus, aplasia and karyomegaly occurring at a low incidence.

The incidence at which tubular basophilia (Fig. 14), was observed was similar in both sexes, with close to 45% of control NZW rabbits showing this change. Likewise, the incidence of mineralization (Fig. 15), was similar between males and females and, in most cases, it was not specified where in the kidney the mineral foci were seen. Nevertheless, in males, the incidence of these foci was similar in different compartments (cortex, cortico-medullary junction, and medulla) whereas in females, a slightly higher incidence was observed in the cortex and corticomedullary junction. Inflammatory changes—namely infiltrates of mononuclear cells, heterophils or mixed cells, and inflammation—were observed in approximately 8% of control NWZ rabbits with similar incidences in both sexes, with mononuclear cell infiltrates being the most common. Tubular dilatation (Fig. 16) was observed at a higher incidence in females than in males, at 15.7% and 3.9%, respectively. Degeneration associated with regeneration was observed at a slightly higher incidence in both sexes compared with degeneration on its own. The incidence at which tubular casts was observed was similar in both sexes, with the hyaline type being observed more frequently than the granular type; however, in some cases, it was not possible to ascertain the type of cast (NOS). The incidence of cysts was relatively low in both sexes, with cortical cysts (Fig. 17), being slightly more frequent than medullary cysts. Cortical scar/fibrosis was observed in both sexes, with a slightly higher incidence in females. Pigment, (Fig. 18), was also observed at a slightly higher incidence in females than in males. All other changes, such as subcapsular hemorrhage/fibrosis, thrombus, pelvic calculus, aplasia, and karyomegaly, were observed at a low incidence and only in females.

In the urinary bladder, mineralization was the most observed spontaneous finding in control NZW rabbits, and it was seen at a higher incidence in males than in females, followed by calculus, which was seen at a slightly higher incidence in females and infiltrate, inflammatory cell (NOS), which was only observed in females.

Discussion

The authors could find no publication of a color atlas of spontaneous histopathology in NZW rabbits. Therefore, microscopic findings in control NZW rabbits in GLP-compliant studies conducted by a laboratory services corporation in the UK and USA over a ten-year period (2011–2020) were presented here with photomicrographs as a reference for the incidence of spontaneous histopathology in NZW rabbits. The authors used terminology published in the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND) publications wherever possible[1]. One challenge in presenting these data lay in combining multiple terms that appeared to represent a single histopathologic entity. For example, although the finding ‘inflammatory cell infiltrates’ does not specify individual cell types and may have been used to record mononuclear cell infiltrates, we kept ‘inflammatory cell infiltrates’ separate from ‘mononuclear cell infiltrates’ with the intention that the former, like

Table 17. Urinary System—Incidence of Microscopic Findings in New Zealand White Rabbits

Tissue/Finding	Male		Female	
	Occurrence/ No. examined	Incidence (%)	Occurrence/ No. examined	Incidence (%)
Kidney				
Aplasia, unilateral	0/203	0.0	1/204	0.5
Basophilia, tubule	86/203	42.4	90/204	44.1
Calculus, pelvis	0/203	0.0	1/204	0.5
Cast, granular	0/203	0.0	2/204	1.0
Cast, hyaline	4/203	2.0	5/204	2.5
Cast, NOS	5/203	2.5	7/204	3.4
Cyst, cortex	2/203	1.0	6/204	2.9
Cyst, medulla	1/203	0.5	2/204	1.0
Cyst, NOS	1/203	0.5	3/204	1.5
Degeneration/regeneration, tubule	7/203	3.4	6/204	2.9
Degeneration, tubule	1/203	0.5	1/204	0.5
Dilatation, tubule	8/203	3.9	32/204	15.7
Hemorrhage/Fibrosis, subcapsular	0/203	0.0	1/204	0.5
Infiltrate, heterophils	0/203	0.0	1/204	0.5
Infiltrate, inflammatory cell, NOS	2/203	1.0	2/204	1.0
Infiltrate, mononuclear cell	15/203	7.4	17/204	8.3
Inflammation, mononuclear cell	1/203	0.5	1/204	0.5
Karyomegaly, collecting ducts	0/203	0.0	1/204	0.5
Mineralization, cortex	6/203	3.0	16/204	7.8
Mineralization, cortico-medullary	6/203	3.0	8/140	5.7
Mineralization, medulla	6/203	3.0	6/204	2.9
Mineralization, NOS	13/203	6.4	13/204	6.4
Mineralization, tubule	11/203	5.4	14/204	6.9
Mineralization, vascular	0/203	0.0	2/204	1.0
Pigment	1/203	0.5	5/204	2.5
Scar/fibrosis, cortex	2/203	1.0	7/204	3.4
Thrombus	0/203	0.0	1/204	0.5
Urinary bladder				
Calculus	3/186	1.6	5/186	2.7
Infiltrate, inflammatory cell, NOS	0/186	0.0	3/186	1.6
Mineralization	13/186	7.0	7/186	3.8

NOS: not otherwise specified.

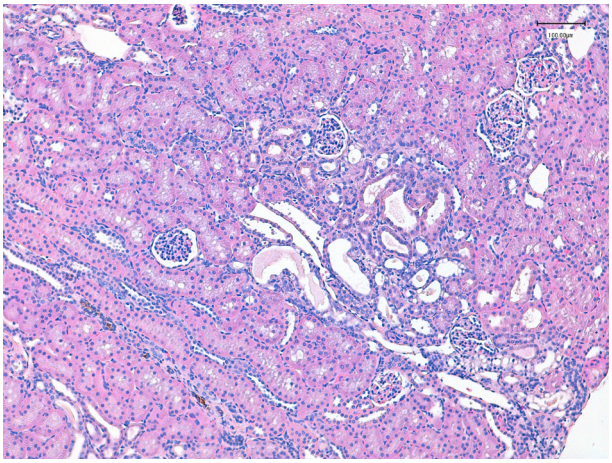

Fig. 14. Tubular basophilia, kidney. 20×, H&E.

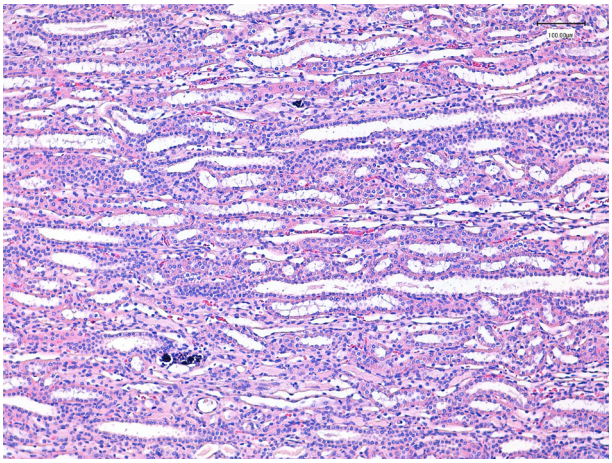

Fig. 15. Mineral, kidney. 10×, H&E.

'mixed cell infiltrates', could include granulocytes (heterophils, basophils or eosinophils).

In the digestive system, musculoskeletal system and endocrine system (specifically, the thyroid), the terms 'inflammation', 'inflammatory cell infiltrates (NOS)', or 'mixed cell' infiltrates were the most reported findings. Although many pathologists do not use the word 'inflammation' unless there are corroborative findings (edema, fibrin, hemorrhage, degeneration, necrosis) in addition to the leukocytes, for the purpose of this project, we decided to keep the word

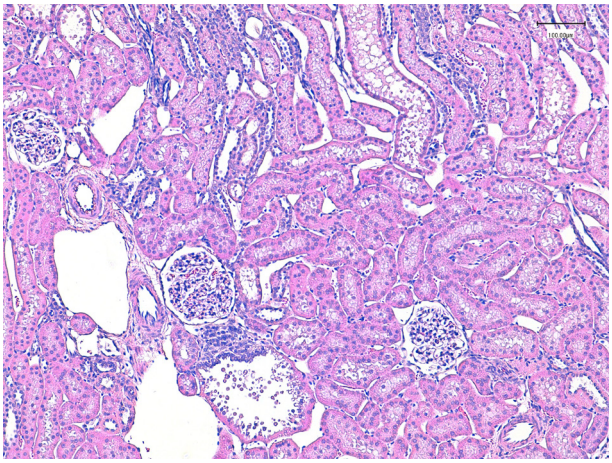

Fig. 16. Tubular dilatation, kidney. 10×, H&E.

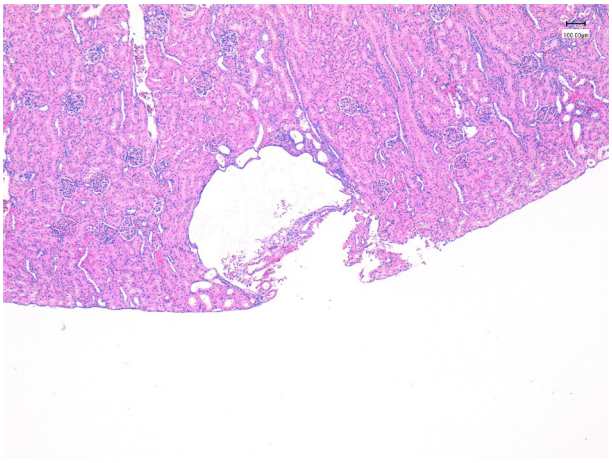

Fig. 17. Cortical cyst, kidney. 4×, H&E.

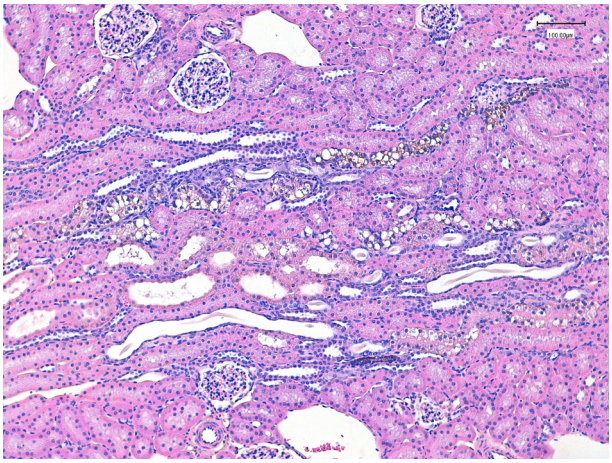

Fig. 18. Tubular epithelial vacuolation and pigment, kidney. 20×, H&E.

'inflammation', when it was recorded in the database, admittedly assuming that sufficient findings were noted (although not reported separately) to merit use of the term.

In the female reproductive system, mineralization (ovary), cysts (oviduct) and increased secretions (mammary gland) were the most reported background findings. In one study of domestic European 'pet' rabbits of the same species, (*Oryctolagus cuniculi*), ovarian lesions were noted in 12 of 44 female rabbits, corroborating the prevalence of ovarian findings but not the type. Ovarian cysts (follicular or of the rete ovarii) were the prevalent finding in that study[13]. Cysts in the mesosalpinx (associated with oviducts) which arise from remnants of the mesonephric duct, have been reported in rabbits, as they have in rodents, but were considered rare in rabbits[13]. In this study cysts of the oviduct were reported in 4.7% of females, therefore common, but ovarian cysts were uncommon (0.5%).

Inflammation (of unspecified type) or mixed inflammatory cell infiltrate were noted in the integument, perianal skin/subcutis or at the site of intramuscular injection and resembled those noted in rodents used in similar types of studies. In rodents, epidermal infiltrates were often associated with dermal inflammation[14], which were not reported in the rabbits in this study.

Findings noted as common in the respiratory system of control rabbits were limited to inflammatory cell infiltrate, mixed cell inflammation (sometimes in alveoli), and hemorrhage in the lung. In rodents, acute inflammation is often associated with other microscopic findings (hyperplasia, epithelial erosion/ulceration) and is usually accompanied by vascular congestion, edema, granulocytes, accumulation of exudates[15]. In rodents, inflammation can be divided into two patterns based on organization of the findings: an alveolar/interstitial pattern or, for lesions originating at terminal airways, a bronchioloalveolar pattern, which helps to determine pathogenesis[15]. A limitation of this study was that localization data of this kind for the lung was not recorded for rabbits.

This study focused exclusively on NZW rabbits used in drug development by one multinational laboratory services provider. A potential weakness of the study was that syndromic data were not entered into the database. Data were entered into the historical control database as individual microscopic findings without correlations between tissues or with macroscopic observations, therefore, conditions such as mucoid enteropathy or parasitic disease were not captured for analysis. 'Abnormal contents' of the gastrointestinal tract were not evaluated, therefore the incidence of, or conditions associated with, clinically observed diarrhea were not captured in this study. This study focused exclusively on histopathologic findings. Findings noted in NZW rabbits between 2011–2020 by one laboratory services provider may not be comprehensive for the species.

Strengths of this study were that a complete tissue list was examined in many of the rabbits. When a tissue was mentioned, the number of animals examined was available and was tabulated when findings were present. Therefore,

the reader can state with confidence that, for a particular tissue, a given number of rabbits was evaluated and a particular finding was or was not present with incidence data. For example, in ten years of historical control data involving the evaluation of 56 rabbits, no abnormalities were found in femur, rib or sternum.

Another strength of the study was that data were collected in the United Kingdom and North America under comparable husbandry conditions, which minimized variables associated with geography, husbandry, diet, and light/dark cycle and thereby increased the confidence possible in the reported data.

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