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Original Research

Spontaneous Ocular Abnormalities in Sprague–Dawley Rats

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We collected historical control data derived from pretreatment ophthalmologic examinations of young (4 to 7 wk of age) Sprague–Dawley (Crl:CD[SD]) male, (2033, 42 lots) and female (1322, 32 lots) rats used in toxicity studies at our facility from 2004 through 2015. Ophthalmologic examination of male and female rats by using a binocular indirect ophthalmoscope and slit lamp revealed high incidences of corneal opacity (61% and 60%, respectively), lenticular opacity (43% and 47%), persistent hyaloid artery (21% and 17%), and retinal folds (27% and 27%). All other ocular abnormalities of the globe, conjunctiva, cornea, anterior chamber, lens, iris, vitreous, and choroid or retina occurred at incidences of less than 5%. Corneal opacities were localized mainly in the corneal nasal (38% and 37%) and paracentral (32% and 33%) areas, and lenticular opacities predominantly occurred in the nuclear area (31% and 34%). We then compared the incidences of spontaneous ocular abnormalities between the first (2004 through 2009) and second (2010 through 2015) 6-y periods. Corneal opacity and persistent hyaloid artery in male and female rats occurred more frequently during the second 6-y than during the first (corneal opacity, second period: male, 68%; female, 66%; corneal opacity, first period: 49% and 51%; persistent artery, second period, 26% and 23%; persistent artery, first period; 12% and 10%). These results support the importance of updating historical control data regularly and providing useful information for toxicologists and ophthalmologists to differentiate treatment-related changes from spontaneous lesions.

Abbreviations: FP, first 6-year period; SP, second 6-year period.

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Albino rats are commonly used in general, nonclinical toxicity studies of chemicals and drugs. In particular, Sprague–Dawley rats are among the most used of the animal strains in many laboratories and facilities, and this strain is particularly suitable for toxicity assessment studies because extensive biologic reference data are available.

Ocular effects due to chemicals and drugs are infrequent safety issues in nonclinical toxicity studies. However, the observation of ocular toxicity can result in the suspension of further compound development or in the regulation of further use of the chemical, to avoid human exposure. In nonclinical in vivo toxicity studies, eyes are routinely evaluated through the observation of clinical signs and performance of ophthalmologic and histopathologic examination, to assess the potential of a drug for inducing ocular toxicity. Morphologic ocular changes are found mainly through ophthalmologic examination using slit-lamp biomicroscopy and direct or indirect ophthalmoscopy.

To appropriately assess ocular toxicities in nonclinical studies, inhouse historical control data are important for understanding normal variations in spontaneous ocular abnormalities in the experimental animals. In general, historical control data are accumulated by each laboratory or facility and used for the assessment of toxicities. Because these data might vary depending on the lot of the animals examined, the laboratories or facilities at which the examinations are conducted, and the data collection period, historical control data should be verified and updated regularly to ensure that they remain valid over time. In addition to the historical control data collected by each laboratory or facility, reports from the literature regarding the incidence of spontaneous ocular abnormalities in Sprague–Dawley rats are available.[2-8,10,11] These published reports are useful in compensating for low numbers of historical control data, especially for ocular abnormalities that occur infrequently.

Here we report the spontaneous ocular abnormalities in 4- to 7-wk-old Sprague–Dawley (Crl:CD[SD]) rats used in the toxicity studies conducted at our facility from 2004 through 2015. The historical control data were derived from 2033 male (42 lots) and 1322 female (32 lots) rats. We also examined whether the incidence of spontaneous ocular abnormalities differed depending on the period of data collection; to this end, we compared the data from 2004 through 2009 (first 6-y period [FP]) with those from 2010 through 2015 (second 6-y period [SP]). Our study provides large-scale data regarding spontaneous ocular abnormalities observed in from multiple lots of Sprague–Dawley rats and shows differences in the incidence of ocular abnormalities depending on the data collection period.

Materials and Methods

Animals and housing conditions. SPF Sprague–Dawley rats (Crl:CD[SD]) (age, 3 to 6 wk) were purchased from Charles River Laboratories Japan (Tsukuba, Atsugi or Hino Breeding Center, Ibaraki, Kanagawa or Shiga, Japan). After quarantine and acclimation periods of 1 to 2 wk, ophthalmologic examinations of 2033 male (42 lots) and 1322 female (32 lots) rats were conducted at our laboratory from 2004 through 2015; at the time

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of the examinations, the animals were 4 to 7 wk old (males) or 4 to 6 wk old (females). The animals were housed individually in cages with bedding (white flakes, Charles River Laboratories Japan) or in stainless steel cages, in an air-conditioned animal room under controlled environmental conditions: room temperature, 23 °C ± 3 °C; relative humidity, 50% ± 20%; and 12:12-h light:dark cycle (lights on, 0715 to 1915). Pelleted diet (MF or CR-LPF, Oriental Yeast, Tokyo, Japan) and ultrafiltered water were available ad libitum.

All experimental procedures were performed with the approval of the IACUC of Taisho Pharmaceutical (Saitama, Japan).

Ophthalmologic examinations. Prior to ophthalmologic examination, a mydriatic agent (tropicamide–phenylephrine hydrochloride, Mydrin-P, Santen Pharmaceutical, Osaka, Japan) was instilled into the rats' eyes to dilate the pupils. A head-worn binocular indirect ophthalmoscope (Omega 200 or 500, Heine Optotechnik, Herrsching, Germany) with a 28-diopter lens and a handheld slit lamp (SL5, SL14, or SL15, Kowa, Aichi, Japan) were used to examine the anterior segments of the eyes, cornea, intermediate optic media, and fundus oculi (Figure 1). During examinations, animals were restrained manually and without anesthesia. All procedures were completed in less than 5 min per animal, and the ophthalmologic findings were judged by board-certified ophthalmologists (Diplomates of the Japanese College of Fundamental Ophthalmologists) or by personnel trained by the ophthalmologists and who had more than 4 y.

Localization and severity of corneal and lenticular opacities. Corneal opacities were classified according to location (Figure 2; superior, inferior, nasal, temporal, paracentral, or endothelial) and severity (slight, mild, moderate, or severe). Lenticular opacities were classified according to location (Figure 3; anterior pole, anterior cortical, anterior suture line, nuclear, nuclear suture line, posterior suture line, posterior cortical, or posterior pole) and size (pinpoint, very small [occurring in less than 1/8 of the area], small [1/8 to 1/4], medium [1/4 to 1/2], large [more than 1/2], or total [entire area]).

Data analysis. The incidences of the spontaneous abnormalities in each site of the eyes in all the animals examined, including males and females, were recorded from all the rats. To examine the sex differences, the incidences of the abnormalities in each site in the males were statistically compared with those in the females.

To assess whether the incidences of spontaneous ocular abnormalities varied depending on when examinations were performed, the data were divided into 6-y collection periods (FP: 2004 through 2009; males, 19 lots; females, 13 lots; SP: 2010 through 2015; males, 23 lots; females, 19 lots). The incidences of abnormalities at each ocular site were compared statistically between data collection periods. The incidences of spontaneous ocular abnormalities were compared between sexes and data collection periods by using the Fisher exact test in SAS (version 9.2, SAS Institute, Cary, NC). The significance level was set at 5% (2-tailed).

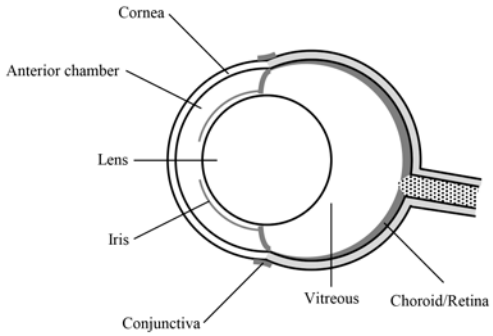

Figure 1. The structures of the eye.

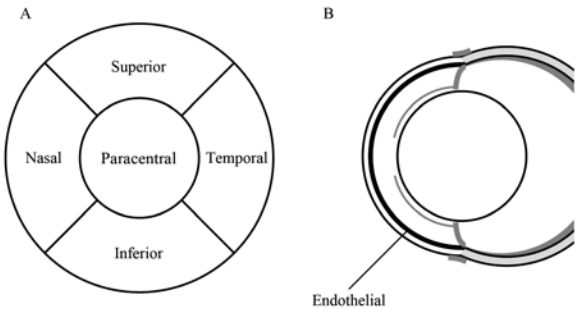

Figure 2. The locations in each area of the cornea, left eye. (A) Transverse plane. (B) Sagittal plane.

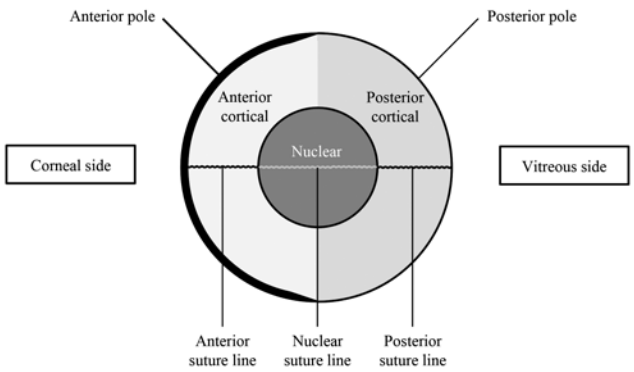

Figure 3. The locations in each area of the lens, sagittal plane.

Results

Incidences of spontaneous ocular abnormalities. The incidences of the spontaneous abnormalities in each site of the eyes in all the rats are shown in Table 1.

The most common abnormalities were opacities in the cornea and lens. The incidences of corneal opacity were 61.1% and 59.8% in male and female rats, respectively, and of lenticular opacity were 43.1% and 47.0%, respectively. Persistent hyaloid artery in the vitreous and retinal folds occurred at relatively high incidence: 20.6% and 17.5% for persistent hyaloid artery in males and females and 27.4% and 26.8% for retinal fold, respectively. The incidences of lenticular opacity and persistent hyaloid artery differed significantly ($P < 0.05$) between male and female rats (Table 1). All other ocular abnormalities (miosis, hyphema, persistent pupillary membrane, posterior synechia, hemorrhage from persistent hyaloid artery and hemorrhage in the vitreous, and hemorrhage and hyperreflectivity in the choroid or retina) occurred at incidences of less than 5%. Abnormalities including enophthalmos, persistent hyperplastic primary vitreous, and coloboma in the choroid or retina were observed at incidences lower than 0.5% (data not shown).

Localization and severity of corneal and lenticular opacities. The localization and severity of corneal and lenticular opacities in all animals are shown in Tables 2 and 3, respectively. In both male and female rats, corneal opacities mainly localized in the nasal and paracentral areas, with severity ranging from slight to moderate (Figure 4). Lenticular opacities occurred predominantly in the nuclear area, were pinpoint to very small in size, and were found in both male and female rats (Figure 5).

Table 1. Spontaneous ocular abnormalities in Sprague–Dawley rats, 2004 through 2015

	Male (n = 2033)	Female (n = 1322)
Not remarkable	277 (13.6%)	184 (13.9%)
Globe		
Miosis	22 (1.1%)	18 (1.4%)
Conjunctiva	0 (0.0%)	0 (0.0%)
Cornea		
Opacity	1243 (61.1%)	790 (59.8%)
Lens		
Opacity	876 (43.1%)	622 (47.0%)[a]
Anterior chamber		
Hyphema	28 (1.4%)	18 (1.4%)
Iris		
Persistent pupillary membrane	34 (1.7%)	19 (1.4%)
Posterior synechia	27 (1.3%)	17 (1.3%)
Vitreous		
Persistent hyaloid artery	419 (20.6%)	231 (17.5%)[a]
Hemorrhage from persistent hyaloid artery	84 (4.1%)	54 (4.1%)
Hemorrhage	39 (1.9%)	20 (1.5%)
Choroid or retina		
Retinal fold	558 (27.4%)	354 (26.8%)
Hemorrhage	57 (2.8%)	30 (2.3%)
Hyperreflectivity	16 (0.8%)	12 (0.9%)

[a]Significant difference ($P < 0.05$, Fisher exact test) between values for male and female rats.

Table 2. Spontaneous corneal opacities in Sprague–Dawley rats, 2004 through 2015

	Slight	Mild	Moderate	Severe	All grades
Male (n = 2033)					
Nasal	174 (8.6%)	467 (23.0%)	130 (6.4%)	0 (0.0%)	771 (37.9%)
Paracentral	114 (5.6%)	283(13.9%)	258 (12.7%)	2 (0.1%)	657 (32.3%)
Temporal	7 (0.3%)	11 (0.5%)	0 (0.0%)	0 (0.0%)	18 (0.9%)
Superior	4 (0.2%)	1 (0.0%)	0 (0.0%)	0 (0.0%)	5 (0.2%)
Inferior	0 (0.0%)	4 (0.2%)	0 (0.0%)	0 (0.0%)	4 (0.2%)
Endothelial	0 (0.0%)	1 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.0%)
Female (n = 1322)					
Nasal	106 (8.0%)	276 (20.9%)	106 (8.0%)	0 (0.0%)	488 (36.9%)
Paracentral	80 (6.1%)	142 (10.7%)	214 (16.2%)	0 (0.0%)	436 (33.0%)
Temporal	3 (0.2%)	2 (0.2%)	2 (0.2%)	0 (0.0%)	7 (0.5%)
Superior	6 (0.5%)	3 (0.2%)	0 (0.0%)	0 (0.0%)	9 (0.7%)
Inferior	2 (0.2%)	1 (0.1%)	0 (0.0%)	0 (0.0%)	3 (0.2%)
Endothelial	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Comparison of spontaneous ocular abnormalities between FP and SP. The incidences of the spontaneous ocular abnormalities during FP and SP are shown in Table 4. In both sexes, the incidences of corneal opacity and persistent hyaloid artery were significantly ($P < 0.05$) higher during SP as compared with FP. The incidences of miosis, hyphema, persistent pupillary membrane, and hemorrhage in the choroid or retina differed significantly ($P < 0.05$) between FP and SP (Table 4).

Discussion

Here we present historical control data from ophthalmologic examinations from 2033 male (42 lots) and 1322 female (32 lots) young Sprague–Dawley (Crl:CD[SD]) rats during the pretreatment period of nonclinical toxicity studies from 2004 through 2015 at our facility. Among the spontaneous ocular abnormalities, the incidences of corneal opacity (mostly in the nasal and paracentral areas), lenticular opacity (mostly in the nuclear area), persistent hyaloid artery, and retinal fold were high, whereas other ocular abnormalities, including miosis, hyphema, persistent pupillary membrane, posterior synechia, hemorrhage from persistent hyaloid artery and hemorrhage in the vitreous, and hemorrhage and hyperreflectivity in the choroid or retina occurred at incidences of 5% or lower.

The incidences of corneal opacities were 61.1% in male rats and 59.8% in female rats. These incidences are comparable with those reported for young rats in previous studies.[2,7,8] In Sprague–Dawley rats, corneal opacities are reported to be influenced by genetic factors, age, environmental conditions of housing, trauma, and infectious disease.[3,7-9,11] However, the mechanism of the onset of corneal opacities in young Sprague–Dawley rats is

Table 3. Spontaneous lenticular opacities in Sprague–Dawley rats, 2004 through 2015

	Pinpoint	Very small	Small	Medium	Large	All sizes
Male (*n* = 2033)						
Anterior pole	25 (1.2%)	40 (2.0%)	2 (0.1%)	3 (0.1%)	0 (0.0%)	70 (3.4%)
Anterior cortical	36 (1.8%)	60 (3.0%)	4 (0.2%)	1 (0.0%)	0 (0.0%)	101 (5.0%)
Anterior suture line	0 (0.0%)	3 (0.1%)	10 (0.5%)	19 (0.9%)	20 (1.0%)	52 (2.6%)
Nuclear	243 (12.0%)	285 (14.0%)	69 (3.4%)	18 (0.9%)	7 (0.3%)	622 (30.6%)
Nuclear suture line	4 (0.2%)	61 (3.0%)	70 (3.4%)	23 (1.1%)	4 (0.2%)	162 (8.0%)
Posterior suture line	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.0%)	1 (0.0%)
Posterior cortical	21 (1.0%)	10 (0.5%)	2 (0.1%)	0 (0.0%)	0 (0.0%)	33 (1.6%)
Posterior pole	1 (0.0%)	8 (0.4%)	12 (0.6%)	6 (0.3%)	1 (0.0%)	28 (1.4%)
Female (*n* = 1322)						
Anterior pole	28 (2.1%)	20 (1.5%)	0 (0.0%)	0 (0.0%)	1(0.1%)	49 (3.7%)
Anterior cortical	15 (1.1%)	38 (2.9%)	10 (0.8%)	0 (0.0%)	0 (0.0%)	63 (4.8%)
Anterior suture line	0 (0.0%)	3 (0.2%)	4 (0.3%)	11 (0.8%)	3 (0.2%)	21 (1.6%)
Nuclear	187 (14.1%)	215 (16.3%)	37 (2.8%)	7 (0.5%)	0 (0.0%)	446 (33.7%)
Nuclear suture line	3 (0.2%)	33 (2.5%)	40 (3.0%)	23 (1.7%)	5 (0.4%)	104 (7.9%)
Posterior suture line	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	1 (0.1%)	2 (0.2%)
Posterior cortical	10 (0.8%)	16 (1.2%)	1 (0.1%)	1 (0.1%)	0 (0.0%)	28 (2.1%)
Posterior pole	0 (0.0%)	3 (0.2%)	1 (0.1%)	0 (0.0%)	1 (0.1%)	5 (0.4%)

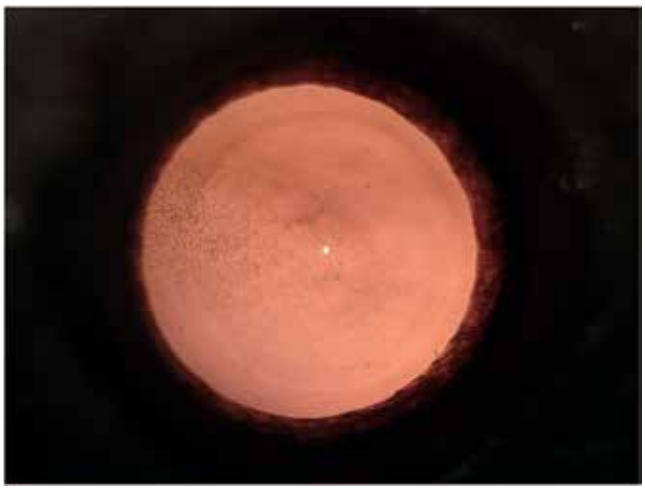

Figure 4. Mild corneal opacity in the nasal area, left eye.

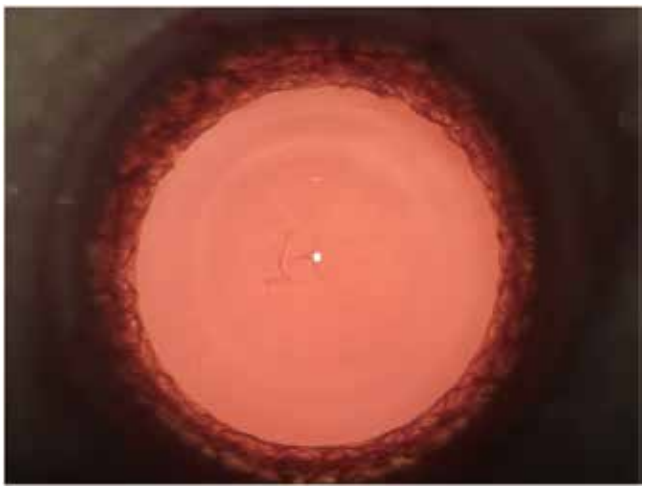

Figure 5. Very small lenticular opacities in the nuclear area.

unknown. Corneal opacities are most frequently observed near the palpebral fissure[8] or in the nasal or paracentral areas. The causes of the high incidences of corneal opacity in these areas are unknown in detail but might reflect prolonged exposure to the external environment. In this study, we conducted a detailed examination of the incidences of corneal opacity in each region of the cornea in Sprague–Dawley rats. The incidences in male and female rats were 37.9% and 36.9% the nasal area, respectively, and 32.3% and 33.0% in the paracentral area.

The incidences of lenticular opacities were 43.1% and 47.0% in male and female rats, respectively. These incidences were comparable to those reported for young rats in previous studies.[2] The incidence of lens opacity reportedly increases with age in Sprague–Dawley rats.[5,7,11] However, in young rats, focal lens opacities can be caused by densification of the nucleus or persistence of a trace of the fetal nucleus.[1] In our historical control data, lenticular opacities predominantly occurred in the nucleus (30.6% and 33.7% in males and females, respectively), ranged in size from pinpoint to very small, and therefore were attributed to densification of the nucleus or persistence of traces of the fetal nucleus. Our data, which showed the highest incidence of lenticular opacities in the nucleus, are consistent with previous reports.[2,7,8]

The incidence of persistent hyaloid artery was 20.6% in male rats and 17.5% in females. Persistent hyaloid artery is frequently observed in young rats and is known to disappear with growth.[5,11] Our values are comparable to those reported for young rats in previous studies.[7,11]

The incidence of retinal folds in our male and female rats was 27.4% and 26.8%, respectively. Retinal folding is a congenital abnormality[10] and is microscopically observed as an abnormal arrangement of the outer nuclear layer. In our historical control data, the incidence of retinal fold was higher than that in previously published reports (0.0% to 1.0%).[6-8] We routinely widely examine the fundus oculi, from the optic disk to the periphery, and detected even small retinal folds. Although the difference in the incidence between this and previous reports might be due to the differences in the criteria for discrimination, our data suggest that retinal folds are a common abnormality in young Sprague–Dawley rats.

In comparisons between the data collection periods, the incidences of corneal opacities and persistent hyaloid artery were higher during SP than FP. Other minor but statistically significant differences between collection periods were observed in the incidences of miosis in the globe, hyphema in the anterior chamber,

Table 4. Comparison of spontaneous ocular abnormalities in Sprague–Dawley rats between first (2004–2009) and second (2010–2015) 6-y periods

	Male		Female	
Group	First 6-y period	Second 6-y period	First 6-y period	Second 6-y period
Collection period	2004–2009	2010–2015	2004–2009	2010–2015
No. of rats examined	781	1252	566	756
Not remarkable	144 (18.4%)	133 (10.6%)[a]	99 (17.5%)	85 (11.2%)[a]
Globe				
Miosis	3 (0.4%)	19 (1.5%)[a]	8 (1.4%)	10 (1.3%)
Conjunctiva	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Cornea				
Opacity	386 (49.4%)	857 (68.5%)[a]	289 (51.1%)	501 (66.3%)[a]
Lens				
Opacity	343 (43.9%)	533 (42.6%)	279 (49.3%)	343 (45.4%)
Anterior chamber				
Hyphema	1 (0.1%)	27 (2.2%)[a]	3 (0.5%)	15 (2.0%)[a]
Iris				
Persistent pupillary membrane	5 (0.6%)	29 (2.3%)[a]	4 (0.7%)	15 (2.0%)
Posterior synechia	6 (0.8%)	21 (1.7%)	8 (1.4%)	9 (1.2%)
Vitreous				
Persistent hyaloid artery	92 (11.8%)	327 (26.1%)[a]	55 (9.7%)	176 (23.3%)[a]
Hemorrhage from persistent hyaloid artery	35 (4.5%)	49 (3.9%)	21 (3.7%)	33 (4.4%)
Hemorrhage	15 (1.9%)	24 (1.9%)	6 (1.1%)	14 (1.9%)
Choroid or Retina				
Retinal fold	209 (26.8%)	349 (27.9%)	151 (26.7%)	203 (26.9%)
Hemorrhage	32 (4.1%)	25 (2.0%)[a]	18 (3.2%)	12 (1.6%)
Hyperreflectivity	8 (1.0%)	8 (0.6%)	8 (1.4%)	4 (0.5%)

[a] Values are significantly different ($P < 0.05$, Fisher exact test) between the first and second 6-y periods.

persistent pupillary membrane in the iris, and hemorrhage in the choroid or retina in one or both sexes. Statistically significant differences between male and female rats also occurred in the incidences of lenticular opacity and persistent hyaloid artery. The incidences of some abnormalities, such as corneal opacities, reportedly are influenced by the housing conditions.[9] However, we did not attribute the differences we noted to housing conditions, because the ratios of cage types used did not differ significantly between FP and SP (87.9% and 89.2% in cages with bedding and 12.1% and 10.8% in stainless steel cages, respectively), and the environmental conditions of housing (room temperature and relative humidity in an air-conditioned animal room) were controlled. The detailed causes of the differences in the incidences of spontaneous ocular abnormalities remain unknown but might be due to differences between lots and in the effects of genetic drift in the animals. These findings indicate that regular verification and updating of historical control data in each laboratory or facility is critical to ensure their experimental validity.

In conclusion, our current results provide useful information for toxicologists and ophthalmologists to assess the ocular toxicities of drugs and chemicals. We recommend that ocular historical control data should be updated regularly at each laboratory or facility.

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