Short Communication

Historical control background incidence of spontaneous pituitary gland lesions of Han-Wistar and Sprague-Dawley rats and CD-1 mice used in 104-week carcinogenicity studies

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Abstract: The aim of this study was to determine the range and incidences of spontaneous microscopic lesions of the pituitary gland in control Han-Wistar and Sprague-Dawley rats and CD-1 mice from 104-week carcinogenicity studies carried out between 1998 and 2010 at Charles River Edinburgh. In both strains of rats and in CD-1 mice, non-proliferative lesions of the pituitary gland were generally uncommon, excluding cysts/pseudocysts (6.42% in Han-Wistar rats, 5.85% in Sprague-Dawley rats, and 2.08% in CD-1 mice). Primary proliferative lesions were most frequently found in the pars distalis of the pituitary gland. Adenomas and carcinomas of the pars distalis were more common in Sprague-Dawley rats (49.33% and 2.85%, respectively) than in Han-Wistar rats (27.29% and 0.21%, respectively), and adenomas in both strains of rats and CD-1 mice exhibited a marked sex predisposition, with females more commonly affected. (DOI: 10.1293/tox.2017-0030; J Toxicol Pathol 2017; 30: 339–344)

Key words: carcinogenicity studies, CD-1, Han-Wistar, pituitary gland, Sprague-Dawley

Proliferative lesions of the pituitary can be induced in rodents by several compounds, irradiation, or high caloric/protein diet[1–3]. Among these compounds, estrogenic agents are well known to cause prolactin (PRL) cell hyperplasia and eventually induce pituitary neoplasia via sustained stimulation with estrogens[4–6]. Compounds which alter the normal pituitary-target organ axis can also secondarily induce proliferative pituitary lesions by hyperstimulatory mechanisms due to altered feedback regulation. For instance, anti-thyroid compounds cause decreased T3/T4 levels in the blood due to disruption of thyroid function, followed by increased thyroid-stimulating hormone (TSH) levels via the negative feedback mechanism of the pituitary-thyroid axis, which result in TSH-producing cell (thyrotroph) hyperplasia[6, 7]. Although proliferative lesions of the pars intermedia are rare compared with those induced in the pars distalis, there are some reports indicating that haloperidol, a dopamine receptor antagonist, can cause diffuse hyperplasia in the pars intermedia[8, 9].

In rodent toxicity studies, spontaneous pituitary neoplasms are frequently encountered[10]. The incidence appears to vary with many factors, including species/strains, age,

sex, and reproductive status[11–13]. Among these factors, it is known that there is a striking degree of strain variation in the incidence of pituitary tumors in rats, which has been reported to range from 10% to more than 90%[3]. Pathological evaluation of lesions caused by xenobiotics must therefore take into account the recognition of background incidences including species, strain, and/or sex differences, and regular updating of the incidence of spontaneous findings is crucial for proper interpretation of drug-induced lesions[14].

The aim of this study was to provide the range and incidences of spontaneous pituitary gland lesions in control rodents from 104-week carcinogenicity studies carried out at Charles River Preclinical Service Edinburgh. Of the different strains of rodents available, Han-Wistar rats, Sprague-Dawley rats, and CD-1 mice are regularly used for carcinogenicity studies in Europe. There are few reports of the incidence and range of pituitary gland findings in these strains[11, 15–17], and most focus only on neoplastic lesions, are of decreasing chronological relevance as historical controls to current populations, or are compiled from a mixture of studies conducted at many facilities in different countries. Here we present data on the incidence of non-proliferative and proliferative findings of the pituitary gland that will serve as a contemporary historical control reference for use in correspondence with regulatory authorities.

In this study, pituitary gland samples from a total of 2,536 rats (1,869 Han-Wistar [Crl:WI(Han)] 667 Sprague-Dawley [Crl:CD(SD)]) and 2,361 CD-1 mice [Crl:CD-1(ICR)] were obtained from control groups of twenty-eight 104-week carcinogenicity studies conducted between 1998 and 2010 at Charles River Edinburgh (Table 1). The ani-

mals were purpose-bred for laboratory use and supplied by Charles River UK Ltd. (Margate, Kent, UK).

Male mice were housed separately, and female mice were housed in groups of up to 3 animals per cage. Rats were housed in groups of up to 5 animals per cage by sex. Animal room temperature and humidity were automatically controlled at 19 to 23°C and 40 to 70%, respectively, with a minimum of 15 air changes/hr. An automatic 12-hr light-dark cycle was maintained. Animals had free access to tap water in bottles with sipper tubes and were fed an *ad libitum* commercial rodent diet (Rat and Mouse [modified] No. 1 Diet SQC Expanded, Special Diet Services Ltd., Witham, Essex, UK). Wooden chewsticks were also offered to all animals for environmental enrichment.

All studies were conducted in accordance with the UK Animals (Scientific Procedures) Act 1986, which conforms to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, Council of Europe).

Animals were humanely euthanized by a rising concentration of carbon dioxide and exsanguinated via femoral veins. Comprehensive necropsy was performed, and tissues were fixed by immersion in 10% neutral-buffered formalin, embedded in paraffin wax, sectioned to a thickness of 4 to 5 µm, mounted onto glass slides, stained with hematoxylin and eosin (H&E), and coverslipped. Data from all studies were recorded by direct computer entry by the study pathologist using PLACES and PLACES 2000 (Instem; Apoloco Limited Systems, Conshohocken, PA, USA). Generally accepted terms were used in the diagnosis of proliferative and non-proliferative lesions (STP/ARP/AFIP SSNDC Guides for Toxicologic Pathology)[2, 18]. All neoplastic findings in each study underwent pathology peer review, and all data were reviewed by the Quality Assurance Department at Charles River's Edinburgh facility prior to the release of the final pathology report.

Statistical analysis of lesion incidence was performed at the 1% significance level (p<0.01) using Fisher's exact test to compare males and females within the same species or strain and to compare different strains of rats (Han-Wistar versus Sprague-Dawley) by sex.

Table 2 presents a summary of pituitary gland non-proliferative findings with their incidences. Overall incidences were not high in either rats or mice.

In rats, non-proliferative lesions of the pituitary gland were more common compared with in mice. Among the findings of rats, cysts/pseudocysts (Fig. 1) were noted in Han-Wistar and Sprague-Dawley rats (6.42% and 5.85%, respectively), and hypertrophy was noted in Han-Wistar rats (2.35%), with the total incidence being higher than 1%.

Table 1. Details of Data Sources

Species/strain	Total number of studies	Route of administration (number of studies)				Total number of animals	
		Oral gavage	Dietary	Subcutaneous injection	Inhalation	Males	Females
Han-Wistar rats	12	5	3	1	3	933	936
Sprague-Dawley rats	4	2	0	1	1	333	334
CD-1 mice	12	7	1	2	2	1,171	1,190

Table 2. Incidence of Spontaneous Pituitary Gland Non-proliferative Lesions in Rats and CD-1 Mice

Findings	Han-Wistar rats			Sprague-Dawley rats			CD-1 mice		
	Males (n = 933)	Females (n = 936)	Total (n = 1,869)	Males (n = 333)	Females (n = 334)	Total (n = 667)	Males (n = 1,171)	Females (n = 1,190)	Total (n = 2,361)
Persistence, Rathke's pouch	0	0	0	1 (0.30%)	0	1 (0.15%)	1 (0.09%)	0	1 (0.04%)
Cysts/pseudocysts[a, b]	82 (8.79%)	38 (4.06%)	120 (6.42%)	15 (4.50%)	24 (7.19%)	39 (5.85%)	36 (3.07%)	13 (1.09%)	49 (2.08%)
Hypertrophy[a]	33 (3.54%)	11 (1.18%)	44 (2.35%)	3 (0.90%)	2 (0.60%)	5 (0.75%)	7 (0.60%)	1 (0.08%)	8 (0.34%)
Hemangiectasis/sinusoidal dilation	8 (0.86%)	10 (1.07%)	18 (0.96%)	0	0	0	0	4 (0.34%)	4 (0.17%)
Hemorrhage/hematoma	0	0	0	1 (0.30%)	0	1 (0.15%)	1 (0.09%)	3 (0.25%)	4 (0.17%)
Cholesterol clefts[a]	11 (1.18%)	0	11 (0.59%)	0	0	0	0	1 (0.08%)	1 (0.04%)
Pigment	2 (0.21%)	0	2 (0.11%)	0	0	0	0	0	0
Vacuolation	1 (0.11%)	1 (0.11%)	2 (0.11%)	0	2 (0.60%)	2 (0.30%)	0	1 (0.08%)	1 (0.04%)
Atrophy	1 (0.11%)	0	1 (0.05%)	0	0	0	0	0	0
Necrosis	1 (0.11%)	1 (0.11%)	2 (0.11%)	0	0	0	2 (0.17%)	0	2 (0.08%)
Extramedullary hematopoiesis	0	1 (0.11%)	1 (0.05%)	0	0	0	0	0	0
Inflammation/inflammatory cell infiltration	0	0	0	1 (0.30%)	0	1 (0.15%)	1 (0.09%)	6 (0.50%)	7 (0.30%)
Arteritis/periarteritis	0	0	0	0	0	0	2 (0.17%)	0	2 (0.08%)
Mineralization	0	0	0	0	0	0	0	1 (0.08%)	1 (0.04%)

Significantly different (p<0.01) incidences of lesions: a = Han-Wistar rats, males vs. females; b = CD-1 mice, males vs. females.

When males and females were compared within the same strain, cysts/pseudocysts and hypertrophy were more frequent in male Han-Wistar rats than in female Han-Wistar rats. In CD-1 mice, the most common non-proliferative lesion was also cysts/pseudocysts, with an incidence of 2.08%, and the incidence of cysts/pseudocysts was significantly higher in males when compared with that in females. The epithelial lining of cysts is generally ciliated, whereas the pseudocyst completely lacks an epithelial lining[18]. Hy-

Fig. 1. Cysts and a pseudocyst are present in the pars distalis and pars intermedia, respectively. Cysts have a ciliated epithelial lining and contain eosinophilic mucoproteinaceous material, whereas the pseudocyst lacks an epithelial lining. Han-Wistar rat. Male. H&E. Bar = 200 μm.

pertrophy was observed mainly in the pars distalis. Other non-proliferative pituitary lesions with a total incidence of below 1% in both rats and mice were persistence of Rathke's pouch, hemangiectasis/sinusoidal dilation, hemorrhage/hematoma, cholesterol clefts, pigment, vacuolation (mainly in the pars distalis), atrophy, necrosis, extramedullary hematopoiesis, inflammation/inflammatory cell infiltration, arteritis/periarteritis, and mineralization. Among them, the incidence of cholesterol clefts in males was higher than that in females when males and females were compared within Han-Wistar rats.

The incidences of proliferative pituitary lesions are summarized in Table 3. Across all studies, neoplastic lesions of the pituitary were more frequently encountered in rats than in mice.

In rats, proliferative lesions of the pars distalis (Fig. 2–4) were overall highly presented in Sprague-Dawley rats compared with Han-Wistar rats. When the 2 strains of rats were compared by sex, the incidences of adenoma and carcinoma of the pars distalis in male and female Sprague-Dawley rats were significantly higher than those in Han-Wistar rats. Females exhibited a higher incidence of hyperplasia in Han-Wistar rats and a higher incidence of adenoma in both strains. In CD-1 mice, hyperplasia and adenoma of the pars distalis was less common than in rats, and no carcinoma were observed. When males and females were compared within CD-1 mice, the incidence of adenoma was higher in females than in males.

Proliferative lesions of the pars intermedia (Fig. 5 and 6) were not frequently encountered either in rats or mice. The
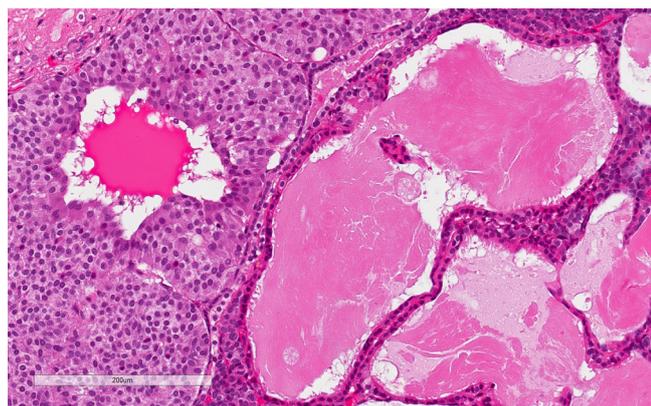
Table 3. Incidence of Spontaneous Pituitary Gland Proliferative Lesions in Rats and CD-1 Mice

Findings	Han-Wistar rats			Sprague-Dawley rats			CD-1 mice		
	Males (n = 933)	Females (n = 936)	Total (n = 1,869)	Males (n = 333)	Females (n = 334)	Total (n = 667)	Males (n = 1,171)	Females (n = 1,190)	Total (n = 2,361)
Pars distalis									
Hyperplasia[c]	240 (25.72%)	310 (33.12%)	550 (29.43%)	89 (26.73%)	79 (23.65%)	168 (25.19%)	26 (2.22%)	36 (3.03%)	62 (2.63%)
Adenoma[a, b, c, d, e]	171 (18.33%)	339 (36.22%)	510 (27.29%)	134 (40.24%)	195 (58.38%)	329 (49.33%)	4 (0.34%)	23 (1.93%)	27 (1.14%)
Carcinoma[a, b]	0	4 (0.43%)	4 (0.21%)	4 (1.20%)	15 (4.50%)	19 (2.85%)	0	0	0
Pars intermedia									
Hyperplasia[b]	26 (2.79%)	20 (2.14%)	46 (2.46%)	6 (1.80%)	0	6 (0.90%)	12 (1.02%)	25 (2.10%)	37 (1.57%)
Adenoma	19 (2.04%)	14 (1.50%)	33 (1.77%)	2 (0.60%)	2 (0.60%)	4 (0.60%)	1 (0.09%)	0	1 (0.04%)
Carcinoma	0	0	0	0	0	0	1 (0.09%)	0	1 (0.04%)
Pars nervosa									
Pituicytoma	0	1 (0.11%)	1 (0.05%)	0	0	0	0	0	0
Ganglioneuroma	0	1 (0.11%)	1 (0.05%)	0	0	0	0	0	0
Tumor of embryonic remnant									
Craniopharyngioma, benign	0	0	0	0	1 (0.30%)	1 (0.15%)	0	0	0
Craniopharyngioma, malignant	1 (0.11%)	0	1 (0.05%)	0	0	0	0	0	0
Secondary tumor									
Lymphoma	4 (0.43%)	3 (0.32%)	7 (0.37%)	3 (0.90%)	0	3 (0.45%)	20 (1.71%)	31 (2.61%)	51 (2.16%)
Leukemia	1 (0.11%)	0	1 (0.05%)	1 (0.30%)	0	1 (0.15%)	6 (0.51%)	3 (0.25%)	9 (0.38%)
Sarcoma	1 (0.11%)	0	1 (0.05%)	0	0	0	0	1 (0.08%)	1 (0.04%)
Schwannoma	1 (0.11%)	0	1 (0.05%)	0	0	0	0	0	0

Significantly different (p<0.01) incidences of lesions: a = males, Han-Wistar rats vs. Sprague-Dawley rats; b = females, Han-Wistar rats vs. Sprague-Dawley rats; c = Han-Wistar rats, males vs. females; d = Sprague-Dawley rats, males vs. females; e = CD-1 mice, males vs. females.
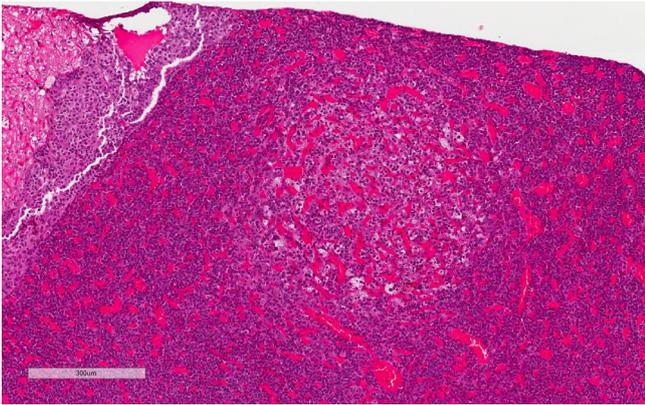
Fig. 2. Hyperplasia, pars distalis. The lesion is not well demarcated and not causing any compression. The cells are slightly enlarged compared with normal surrounding cells. Han-Wistar rat. Male. H&E. Bar = 300 μm.
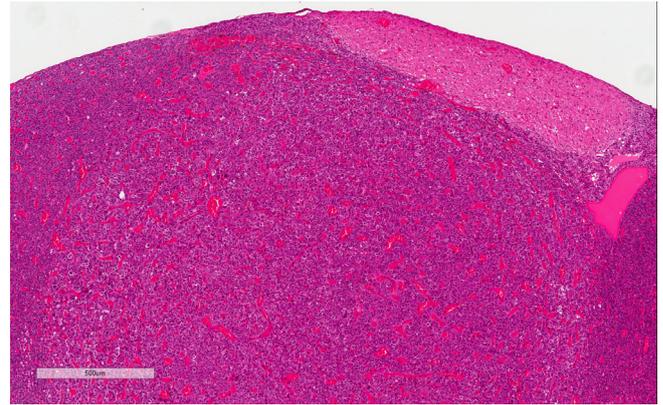

Fig. 3. Adenoma, pars distalis. Compression of the surrounding parenchyma is present. Han-Wistar rat. Female. H&E. Bar = 500 μm.
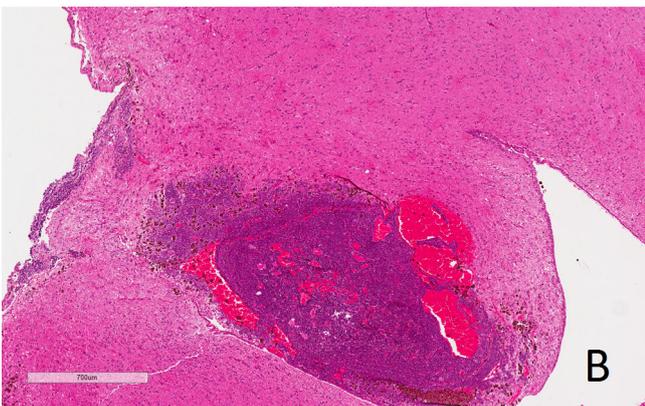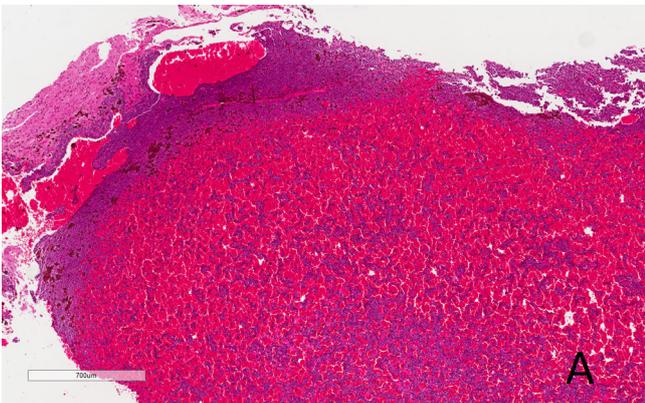

Fig. 4. Carcinoma, pars distalis (A). An invasion of the brain is found (B). Han-Wistar rat. Female. H&E. Bar = 700 μm.
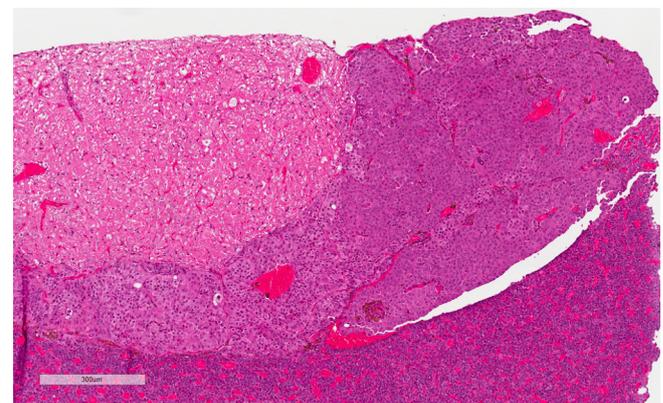

Fig. 5. Hyperplasia, pars intermedia. An increase in number of cells is seen. No compression is present. Han-Wistar rat. Male. H&E. Bar = 300 μm.
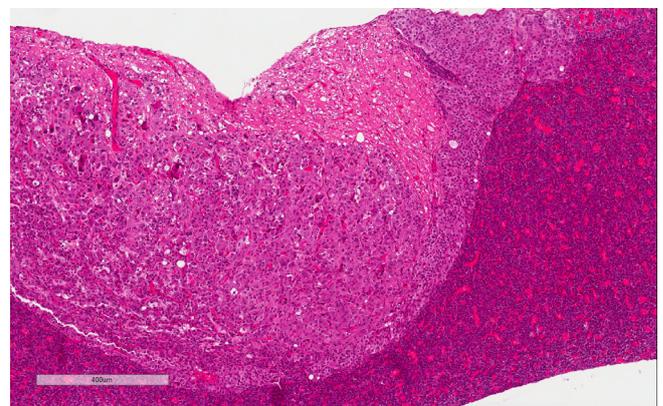

Fig. 6. Adenoma, pars intermedia. The tumor extends into the adjacent pars nervosa. Han-Wistar rat. Male. H&E. Bar = 400 μm.

incidences of hyperplasia and adenoma in Han-Wistar rats (2.46% and 1.77%, respectively) were relatively higher than those in Sprague-Dawley rats (0.90% and 0.60%, respectively), although a statistically significant difference was noted only for hyperplasia in females. No carcinoma were observed in this study. In CD-1 mice, hyperplasia of the pars intermedia was noted with an incidence of 1.57%. Adenoma and carcinoma was less common than hyperplasia, and both were observed with an incidence of below 1%. Other primary tumors noted with an incidence of below 1% in both rats and mice were pituicytoma, ganglioneuroma, and craniopharyngioma. Lymphoma was the most common secondary tumor in rats and mice of both sexes. In CD-1 mice, lymphoma was observed with an incidence of 2.16%. Other secondary tumors noted with an incidence of below 1% in both rats and mice were leukemia, sarcoma, and schwannoma.

As reported by previous studies, spontaneous proliferative lesions of the pituitary gland are more common in rats than mice[19]. In rats, the frequency increases with age in all strains, and the tumors usually begin to appear between 13 and 24 months of age[6]. Primary proliferative lesions of the pituitary are, as is well known, most frequently found in the pars distalis and are rarely encountered in the pars intermedia or pars nervosa[20–23]. Immunohistochemical studies confirmed that most pituitary tumors are PRL positive[24–26]. These reports are consistent with the fact that plasma PRL levels increase with age in rats due to reduced hypothalamic dopamine drive and correlate well with the age-dependent increase in pituitary hyperplasia and tumors observed in this species[27]. Some papers also indicate that proliferative lesions of the mammary gland can be caused by spontaneous pituitary tumors, since PRL is known to have a trophic effect for mammary tumor formation in rats[28, 29]. In the present study, the incidences of adenoma and carcinoma of the pars distalis in Sprague-Dawley rats (49.33% and 2.85%, respectively) were higher than those in Han-Wistar rats (27.29% and 0.21%, respectively). This strain difference may have caused a well-known high prevalence of mammary tumors in Sprague-Dawley rats[30]. This speculation was supported by this study, in which the incidences of mammary tumors were 10.70% in Han-Wistar rats (males, 0.11%, and females, 21.26%) and 39.28% in Sprague-Dawley rats (males, 2.10%, and females, 76.35%), respectively. Also, both strains of rats and the CD-1 mice exhibited a marked sex predisposition, with females more commonly affected for adenoma and/or carcinoma of the pars distalis, which is in line with the previous literature[6]. This higher incidence of pituitary tumors in females suggests that estrogen is involved, either by a direct effect on the pituitary gland or inhibition of dopamine[27]. In mice, it is known that spontaneous pituitary tumors are observed with advancing age at a lower incidence than that in rats and that these tumors occur more often in females than males, and this was confirmed in this study[19, 27]. Similar to the rat, mouse pituitary tumors predominantly arise from PRL cells of the pars distalis[19].

The distinction between adenoma and carcinoma of the pituitary gland is usually based on histological evidence of the absence or presence of local infiltration or invasion of adjacent structures, notably into brain tissue or sphenoid bone[2]. In the present study, however, the gland was removed from the sphenoid bone due to pituitary weight requirements. Therefore, local invasion to the bone may have been overlooked. Thus, we must take into consideration the fact that the incidence of pituitary carcinoma could have been higher if the gland was collected with the surrounding tissues[31].

To the best of our knowledge, this is the most comprehensive combined study of the incidences of background lesions in the pituitary glands in control rats and CD-1 mice. References to the incidences reported here should facilitate the differentiation of spontaneous lesions from induced lesions in toxicological safety studies in these strains of rodents.

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