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AMY E. BRIX,[1] ABRAHAM NYSKA,[2] JOSEPH K. HASEMAN,[3] DONALD M. SELLS,[4] MICHEAL P. JOKINEN,[5] AND NIGEL J. WALKER[6]

[1]*Experimental Pathology Laboratories, Research Triangle Park, North Carolina 27709, USA*
[2]*Laboratory of Experimental Pathology, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina 27709, USA*
[3]*Biostatistics Branch, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina 27709, USA*
[4]*Battelle Columbus Laboratories, Columbus, Ohio 43201, USA*
[5]*Pathology Associates—A Charles River Company, Durham, North Carolina 27713, USA, and*
[6]*Laboratory of Computational Biology and Risk Analysis, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina 27709, USA*

ABSTRACT

The NTP has a long history of using Fischer rats and has compiled a large database of incidences of lesions seen in control animals. Such a database is lacking for Harlan Sprague–Dawley (SD) rats. The intention of this paper is to report spontaneous lesions observed in female vehicle control Harlan SD rats, and to compare the incidence in 2 strains of rats (Fischer and Harlan SD) used in NTP studies. Female Harlan SD rats served as the test animals for a special series of 2-year studies. Male rats were not used in these studies. Complete histopathology was performed on all animals, and the pathology results underwent comprehensive NTP pathology peer review. The most commonly observed neoplasms in these female control Harlan SD rats were mammary gland fibroadenoma (71%), tumors of the pars distalis of the pituitary (41%) and thyroid gland C-cell tumors (30%). Female Fischer rats had incidences of 44% for mammary gland fibroadenomas, 34% for tumors of the pars distalis, and 16% for thyroid gland C-cell tumors. Fischer rats had a 15% incidence of clitoral gland tumors, while the Harlan SD rats had an incidence of <1%. In contrast to Fischer F344 rats, the Harlan SD rats had a high incidence of squamous metaplasia of the uterus (44.2%). Squamous metaplasia is not a lesion commonly observed in NTP control Fischer rats. The Harlan SD rats had a very low incidence of mononuclear cell leukemia (0.5%), compared with an incidence of 24% in female Fischer rats.

Keywords. Spontaneous Lesions; Harlan SD rats; 2-year study.

INTRODUCTION

The National Toxicology Program (NTP) conducted a series of 2-year bioassays in female Harlan Sprague–Dawley (SD) rats to evaluate the chronic toxicity and carcinogenicity of dioxin-like compounds (DLCs).

Harlan SD rats were selected for theses studies, rather than the Fischer 344 rat usually used in NTP chronic studies for 2 reasons: they were the strain of rat used in previous studies with DLCs, and it was hoped that the interpretation of complex hepatic lesions would be simplified by choosing a strain of rat with a lower incidence of mononuclear cell leukemia.

Historical control incidences are important considerations when interpreting data from any study (Haseman et al., 1984). The NTP has a long history of using Fischer rats and has compiled a large database of incidences of lesions seen in control animals. This database is often referred to when the incidence of a lesion in the concurrent control group is outside the historical control range, or to provide support for the significance, or lack thereof, of very uncommon lesions (Deschl et al., 2002; Greim et al., 2003). However, the NTP did not have the breadth of data collected on Harlan SD rats; therefore, this report summarizes the changes found in the female vehicle control animals used in 7 chronic studies. The intention of this paper is to report lesions observed in control Harlan SD rats, and to compare the 2 strains of rats (Fischer and Harlan SD) used in NTP studies. Lesions in other strains of SD rats have been described elsewhere (McMartin et al., 1992; Pettersen et al., 1996).

MATERIALS AND METHODS

The animal studies were carried out under contract at Battelle Columbus Laboratories (Columbus, OH). All animals were under the supervision of a veterinarian and housed in an AAALAC accredited facility for the duration of this study, and animal handling and husbandry was conducted in accordance with NIH guidelines. Female Harlan SD rats, approximately 40 days old, were acquired from Harlan Sprague–Dawley, Inc., Indianapolis, IN, and quarantined for 15 days after being received by the study laboratory, during which time a veterinarian inspected the animals, tested them, and determined them to be free of overt disease. Serum samples were taken for viral screening after the quarantine period and at the study termination.

Address correspondence to: Amy E. Brix, Experimental Pathology Laboratories, Inc., P.O. Box 12766, Research Triangle Park, North Carolina 27709, USA; e-mail: brix@niehs.nih.gov

Abbreviations: DLCs, dioxin-like compounds; NBF, neutral buffered formalin; NTP, National Toxicology Program; SD, Sprague–Dawley.

Animals were randomized and assigned to dose groups, permanently identified with tail tattoos, and group housed, 5 to a cage, in solid polycarbonate cages (Lab Products, Inc., Maywood, NJ). The animals were given irradiated NTP-2000 pelleted feed (Zeigler Bros., Inc., Gardners, PA) and water ad libitum, and kept in rooms maintained between 69° and 75°F, with a relative humidity of 35% to 65%, and 10 changes of filtered air per hour. Control animals were given corn oil:acetone vehicle (99:1 mixture) by gavage 5 days a week for up to 2 years. Animals were observed twice daily for clinical signs of toxicity. Animals in a moribund condition and animals at all scheduled sacrifices were euthanatized by carbon dioxide. Complete necropsies were performed on all animals, including early death animals, using standardized methodology. All masses and morphologic abnormalities, and all protocol-required tissues, were collected at necropsy and placed in 10% neutral buffered formalin (NBF) until processed. Lungs were instilled with 10% NBF prior to being placed in the fixative. The maxillae, including the nose, were decalcified in a 5% Nitric Acid Decal solution for 3 days (Poly Scientific, Bay Shore, NY). Tissues were then trimmed, dehydrated, cleared, and infiltrated with paraffin, embedded, sectioned into 5 μm thick sections, mounted onto glass slides, and stained with hematoxylin and eosin stain.

Complete histopathology was performed and the pathology results underwent comprehensive NTP pathology peer review (Boorman et al., 2002). To enhance comparison of the results of these 7 chronic studies, it was important to maintain consistency both within and between studies. In order to do this, the same pathologists were involved in all phases of the pathology evaluation including the initial examination and the pathology peer review.

Fifty-three female rats were used in each control group for the core study, for a total of 371 animals. Core study animals were sacrificed when moribund or after 2 years of exposure. Additional animals were included in each dose group for interim sacrifices performed at 14 weeks (10 additional animals per group per study for a total of 70 control animals), 31 weeks (10 additional animals per group per study for a total of 70 control animals), and 53 weeks (8 additional animals per group per study for a total of 56 control animals).

RESULTS AND DISCUSSION

A summary of lesions, with their incidences, found in the core study animals can be found in Table 1. Table 2 contains the incidences of lesions seen in the interim sacrifices (14-, 31-, and 53-weeks). There are data from only 1 gavage study in Fischer rats fed the NTP-2000 diet so for comparison purposes, incidences of lesions in female Fischer rats from control groups in feed studies, fed NTP-2000 pelleted feed, were used whenever possible (Haseman et al., 2003). These studies were conducted at either Southern Research Institute in Birmingham, AL, or at Battelle Columbus Laboratories in Columbus, OH. It has been shown previously that there is no difference in the incidences of neoplasms in untreated female rats when compared to those given corn oil by gavage, as the control rats in these studies were given (Boorman et al., 1990). When published incidences of lesions were not available for female Fischer rats fed the NTP-2000 diet, published incidences from control female Fischer rats used in corn oil gavage studies and fed the NIH-07 diet were used (Boorman et al., 1990).

Survival

The average survival in these 7 studies was 41.5%, compared with an average survival rate of 76% for the female control Fischer rats used in 5 chronic NTP feed studies and given the NTP-2000 diet. (Haseman et al., 2003). Two of the studies using Harlan SD rats had survival rates of 28.3% and 30.2%; without these 2 studies, the average survival was 46.4%. There were a total of 3 dosing accidents between the 7 studies; had these 3 animals lived to the end of the study, the average survival would have been 42.3%. The average survival rate of female Fischer rats used in corn oil gavage studies and fed NIH-07 diet was 46.0% (Tumor Incidence in Control Animals by Route and Vehicle of Administration, F344/N Rats, unpublished report, Dec. 1999).

The mean life span of the Harlan SD rats was 628.7 days, while that of Fischer rats used in 5 chronic NTP feed studies and given the NTP-2000 diet was 707 days (Haseman et al., 2003). The mean life span for female Fischer rats used in corn oil gavage studies and fed NIH-07 diet was 674 days (Tumor Incidence in Control Animals by Route and Vehicle of Administration, F344/N Rats, unpublished report, Dec. 1999).

Body Weights

The maximum mean weekly body weight for female Harlan Sprague–Dawley rats used in these 7 studies was 387.3 grams, compared with 320 grams for control female Fischer rats used in feed studies and given NTP-2000 diet (Haseman et al., 2003). The maximum mean weekly body weight for female Fischer rats used in corn oil gavage studies and fed NIH-07 diet was 319.3 grams (Tumor Incidence in Control Animals by Route and Vehicle of Administration, F344/N Rats, unpublished report, Dec. 1999).

Adrenal Cortex

The majority of animals in the core study (81%) had focal hypertrophy of the adrenal cortex. This lesion consisted of enlarged eosinophilic or vacuolated cells in the cortex. Some of the lesions were compressive. Hypertrophy was recorded as early as 14 weeks, and by 53 weeks was recorded in over half of the animals (51.8%). Cystic degeneration, consisting of cystic spaces filled with proteinaceous fluid with variable numbers of erythrocytes, occurred in 18.7% of the animals in the core study. In most instances, the cystic change was within a focus of enlarged eosinophilic or vacuolated cells, and appeared to be a morphologic continuum with hypertrophy. Adrenal cortical hyperplasia, while less common than hypertrophy, was recorded in 30.9% of the animals in the core study. Hyperplasia consisted of a focal increase in the number of cells; the cells were typically smaller and more basophilic than normal cortical cells. Despite the frequency of hypertrophy and hyperplasia in the adrenal cortex, cortical adenomas and carcinomas were uncommon, each occurring only in 0.5% of the animals.

Adrenal Medulla

Hyperplasia of the adrenal medulla was a common lesion, with an incidence of 25.5%. Benign pheochromocytomas

TABLE 1.—Incidences of lesions observed in vehicle control female Harlan Sprague-Dawley core study rats

Organ, lesion	Incidence*	Organ, lesion	Incidence*
Adrenal cortex		Mesentery	
Hypertrophy	81% (299/369)	Schwannoma malignant	0.3% (1/371)
Hyperplasia	30.9% (114/369)	Multiple Organs	
Degeneration, cystic	18.7% (69/369)	Mononuclear cell leukemia	0.5% (2/371)
Adenoma	0.5% (2/369)	Malignant lymphoma	1.1% (4/371)
Carcinoma	0.5% (2/369)	Oral Mucosa	
Adrenal medulla		Gingival, squamous cell carcinoma	1.1% (4/371)
Hyperplasia	25.5% (94/368)	Ovary	
Pheochromocytoma, benign	7.6% (28/368)	Luteoma	0.5% (2/367)
Pheochromocytoma, complex	0.3% (1/368)	Granulosa cell tumor malignant	0.5% (2/367)
Pheochromocytoma, malignant	0.3% (1/368)	Cystadenoma	0.3% (1/367)
Bone		Pancreas, Acinus	
Osteosarcoma	0.3% (1/371)	Atrophy	3.8% (14/366)
Bone Marrow		Hyperplasia	3.0% (11/366)
Lipoma	0.3% (1/371)	Adenoma	0.3% (1/366)
Brain		Parathyroid Gland	
Astrocytoma, malignant	0.5% (2/371)	Adenoma	0.3% (1/336)
Oligodendroglioma, malignant	0.3% (1/371)	Pituitary Gland	
Medulloblastoma, malignant	0.3% (1/371)	Pars distalis, hyperplasia	34.1% (126/369)
Clitoral Gland		Pars intermedia, hyperplasia	0.8% (3/369)
Duct, cyst	72.9% (269/363)	Pars distalis, adenoma	41.2% (152/369)
Adenoma	0.3% (1/363)	Pars distalis, carcinoma	0.3% (1/369)
Ear		Pars intermedia, adenoma	1.1% (4/369)
Pinna, neural crest tumor	0.3% (1/371)	Skeletal Muscle	
Forestomach		Fibrous histiocytoma	0.3% (1/371)
Squamous cell papilloma	0	Skin/Subcutaneous Tissue	
Squamous cell carcinoma	0.5% (2/371)	Basal cell carcinoma	0.3% (1/371)
Heart		Fibroma	1.4% (5/371)
Cardiomyopathy	28.5% (105/369)	Fibrosarcoma	0.3% (1/371)
Schwannoma, malignant	0.8% (3/369)	Sarcoma	0.3% (1/371)
Hemangiosarcoma	0.3% (1/369)	Schwannoma malignant	0.3% (1/371)
Intestine Large, Cecum		Keratoacanthoma	0.3% (1/371)
Leiomyoma	0.3% (1/371)	Squamous cell papilloma	0.3% (1/371)
Intestine Large, Colon		Trichoepithelioma	0.3% (1/371)
Carcinoma	0.3% (1/371)	Thymus	
Intestine Small, Jejunum		Thymoma, benign	0.3% (1/360)
Leiomyosarcoma	0.3% (1/371)	Thyroid Gland	
Fibrosarcoma	0.3% (1/371)	C-cell hyperplasia	31.3% (115/367)
Islets, Pancreatic		C-cell adenoma	26.2% (96/367)
Adenoma	1.6% (6/367)	C-cell carcinoma	3.8% (14/367)
Carcinoma	0.5% (2/367)	Follicular cell adenoma	0.5% (2/367)
Kidney		Tooth	
Nephropathy	61.1% (226/370)	Periodontal tissue, fibrosarcoma	0.3% (1/371)
Transitional epithelium hyperplasia	5.7% (21/370)	Periodontal tissue, neurofibrosarcoma	0.3% (1/371)
Mineralization	75.1% (278/370)	Urinary Bladder	
Hemangiosarcoma	0.3% (1/370)	Papilloma	0.3% (1/367)
Nephroblastoma	0.5% (2/370)	Uterus	
Lipoma	0.3% (1/370)	Metaplasia, squamous	44.2% (164/371)
Renal tubular adenoma	0.3% (1/370)	Endometrium, hyperplasia, cystic	59.3% (220/371)
Renal tubular carcinoma	0.3% (1/370)	Adenoma	0.5% (2/371)
Liver		Carcinoma	0.5% (2/371)
Bile duct hyperplasia	7.8% (29/371)	Fibroma	0.3% (1/371)
Basophilic focus	32.4% (120/371)	Leiomyoma	0.3% (1/371)
Clear cell focus	11.1% (41/371)	Leiomyosarcoma	0.3% (1/371)
Eosinophilic focus	24.0% (89/371)	Polyp stromal	15.6% (58/371)
Mixed cell focus	52.3% (194/371)	Sarcoma stromal	0.3% (1/371)
Hepatocellular adenoma	1.3% (5/371)	Squamous cell papilloma	0.5% (2/371)
Hemangioma	0.3% (1/371)	Squamous cell carcinoma	0.3% (1/371)
Lung		Schwannoma, malignant	0.3% (1/371)
Alveolar epithelium, hyperplasia	28.1% (104/370)	Cervix, schwannoma malignant	0.5% (2/371)
Infiltration cellular, histiocyte	80.3% (297/370)	Cervix, carcinoma	0.3% (1/371)
Alveolar/bronchiolar adenoma	0.5% (2/370)	Vagina	
Lymph Node, Mesenteric		Sarcoma	0.3% (1/371)
Hemangiosarcoma	0.3% (1/371)	Squamous cell papilloma	0.3% (1/371)
Mammary Gland		Squamous cell carcinoma	0.3% (1/371)
Hyperplasia	50.9% (189/371)	Malignant schwannoma	0.3% (1/371)
Adenoma	3.0% (11/371)	Zymbal's Gland	
Carcinoma	11.3% (42/371)	Carcinoma	0.3% (1/371)
Fibroadenoma	70.9% (263/371)		

*Incidence defined as the number of animals with the lesion divided by the number of animals with the tissue examined histologically, multiplied by 100. The denominator varies between tissues due to various causes, including autolysis and tissues not identified at necropsy or trimming.

were much less common, occurring in only 7.6% of the animals. Pheochromocytomas tended to be larger than hyperplasia and were distinguished from hyperplasia based on the former showing distinct compression of the medulla and/or cortex. Only 1 malignant pheochromocytoma (incidence 0.3%) and 1 complex pheochromocytoma (incidence 0.3%) were recorded. A pheochromocytoma was considered malignant if it showed distinct invasion through the

TABLE 2.—Incidences of lesions observed in vehicle control female Harlan Sprague–Dawley rats at interim sacrifices.

Organ, lesion	Incidence		
	14-week interim sacrifice	31-week interim sacrifice	53-week interim sacrifice
Adrenal Cortex			
Hypertrophy	7.1% (5/70)	27.1% (19/70)	51.8% (29/56)
Hyperplasia		1.4% (1/70)	7.1% (4/56)
Degeneration, cystic		1.4% (1/70)	7.1% (4/56)
Adrenal Medulla			
Hyperplasia		1.4% (1/70)	
Clitoral Gland			
Carcinoma			1.8% (1/56)
Liver			
Basophilic focus		1.4% (1/70)	14.3% (8/56)
Clear focus			7.1% (4/56)
Eosinophilic focus		2.9% (2/70)	5.4% (3/56)
Mixed focus		32.9% (23/70)	62.5% (35/56)
Lung			
Infiltration cellular, histiocyte	1.4% (1/70)	14.3% (10/70)	53.6% (30/56)
Mammary Gland			
Hyperplasia		4.3% (3/70)	10.7% (6/56)
Fibroadenoma		1.4% (1/70)	
Carcinoma	1.4% (1/70)		
Pancreas, Acinus			
Hyperplasia			1.8% (1/56)
Atrophy	1.4% (1/70)	7.1% (5/70)	
Pituitary Gland			
Pars distalis, Hyperplasia		2.9% (2/70)	5.4% (3/56)
Skin			
Sarcoma		1.4% (1/70)	
Thyroid Gland			
c-cell hyperplasia			14.3% (8/56)
c-cell adenoma			8.9% (5/56)
Uterus			
Metaplasia, squamous	4.3% (3/70)	62.9% (44/70)	87.5% (49/56)
Endometrium, Hyperplasia, cystic	24.3% (17/70)	24.3% (17/70)	58.9% (33/56)
Polyp stromal			1.8% (1/56)

capsule of the adrenal gland or into a blood vessel. Complex pheochromocytoma was used when a tumor had a substantial neural and neuronal component. The incidences of adrenal medullary tumors were similar to those previously reported in Harlan SD rats, 11% for benign medullary tumors and 0% for malignant medullary tumors (Kaspareit and Rittinghausen, 1999). The historical incidence for adrenal medullary tumors in female Fischer rats used by the NTP is 3% (Haseman et al., 2003).

Clitoral Gland

Cysts of the ducts of clitoral glands were recorded in 72.9% of the animals in the core study. These cysts consisted of dilated ducts that were filled with keratin and lined by attenuated epithelium. The Fischer rat data base terms this change "ductal ectasia of the clitoral glands," and it is also a common finding in old Fischer rats (Boorman et al., 1990). Tumors of clitoral gland were rare in Harlan SD rats, with only 1 being recorded (incidence 0.3%). This is in contrast to their relatively high incidence in Fischer rats, where they had an incidence of 15% (Haseman et al., 2003).

Heart

Over one quarter (28.5%) of the Harlan SD animals had cardiomyopathy recorded at the end of the chronic studies. This is a common spontaneous lesion in aging Fischer rats as well (Boorman et al., 1990). Cardiomyopathy in Harlan SD rats was typically of minimal severity and similar to that seen in Fischer rats, consisting of hypereosinophilic myofibers that lacked cross-striations; vacuolated, granular, degenerative myofibers; infiltrations of mononuclear cells; separation of myofibers by myxomatous material; and eventual replacement of myofibers by fibrous connective tissue. Three animals had malignant schwannomas in the heart, and 1 hemangiosarcoma was present.

Kidney

Nephropathy (chronic progressive nephropathy) was recorded in 61.1% of core study animals. This represents a lower incidence than that seen in female Fischer rats (75%) fed the NTP 2000 diet (Haseman et al., 2003). Nephropathy present in the Harlan SD rats was morphologically similar to that seen in Fischer rats (Boorman et al., 1990) and consisted of basophilic, regenerative tubules advancing to dilated tubules filled with proteinaceous casts and surrounded by fibrous connective tissue and inflammatory cells. Mineralization was also very commonly seen in the kidneys of these Harlan SD rats. Twenty-one animals (5.7%) had hyperplasia of the transitional epithelium, which was characterized by an increased number of layers and small papillary projections of transitional epithelium.

Nephroblastoma was the most commonly recorded neoplasm in the kidney with an incidence of 0.5%. Single occurrences of hemangiosarcoma, lipoma, renal tubular adenoma, and renal tubular carcinoma were also present. These tumors also have a very low incidence in Fischer rats (Boorman et al., 1990).

Liver

Foci of hepatocellular alteration were common in control female Harlan SD rats. Mixed cell foci were the most common, observed in over half (52.3%) of the core study animals. These foci were usually small and composed of a combination of clear cells and eosinophilic cells. Less commonly, they consisted of a combination of clear cells and basophilic cells. It was not uncommon for an animal to have more than one mixed cell focus. Many of these lesions might have been called "clear cell foci" if the diagnostic term "mixed cell foci" had not been available. Basophilic foci and eosinophilic foci were also common, with incidences of 32.4% and 24.0%, respectively. Basophilic foci contained small hepatocytes with cytoplasm that was slightly more basophilic when compared to surrounding hepatocytes. Eosinophilic foci were discrete areas containing cells that were typically larger than surrounding hepatocytes with brightly eosinophilic cytoplasm.

Clear cell foci were the least common type of focus recorded, occurring in 11.1% of the core study animals. These cells had a clearing of the cytoplasm similar to that seen with glycogen accumulation. The cells tended to be normal size and the foci were typically small. Foci were recorded in the liver as early as 31 weeks. At this time point, mixed cell foci were common, being recorded in 32.9% of the animals. This incidence increased to 62.5% at 53 weeks, at which time basophilic foci, clear cell foci, and eosinophilic foci were also observed. In female Fischer rats, the most common type of focus was the basophilic focus, with a 46% incidence at 6 months, and a 100% incidence by 24 months.

Clear cell foci were the next most frequently recorded (69% incidence by 24 months), with vacuolated, eosinophilic, and mixed cell foci being less common (31, 29, and 28% incidence at 24 months, respectively) (Boorman et al., 1990).

Five hepatocellular adenomas were present in core study rats for an incidence of 1.3%. Hepatocellular adenomas tended to be larger than foci, caused compression of surrounding hepatocytes, and often distorted the normal capsular surface of the liver. Normal hepatic lobular architecture was missing, and often there was slight-to-moderate cellular atypia. Mitoses were not usually observed. A single hemangioma was observed in the liver.

Bile duct hyperplasia was present in 29 animals (7.8% incidence). In control female rats, these lesions typically consisted of increased profiles of bile ducts in the portal area, surrounded by thin bands of fibrous connective tissue.

Lung

Histiocytic infiltration (alveolar histiocytosis) was common in lungs of control animals, and was observed, in 1 animal, as early as 14 weeks. By the end of 2 years, this lesion was seen in 80.3% of the animals. It consisted of clusters of foamy macrophages within alveolar spaces, often at the tip of a lung lobe, or just below the pleura. Alveolar epithelial hyperplasia was present in 28.1% of the animals by the end of 2 years. These lesions were typically associated with infiltrates of histiocytes or other inflammatory cells, in contrast to foci of alveolar epithelial hyperplasia that appears preneoplastic. Alveolar/bronchiolar adenomas were present in 2 core study animals (incidence, 0.5%). Alveolar/bronchiolar adenomas and carcinomas had a combined incidence of 3% in female Fischer rats (Haseman et al., 2003).

Mammary Gland

Fibroadenomas were the most frequently occurring mammary gland tumor in the Harlan SD rats, observed in 70.9% of the core study animals. Hyperplasia of the mammary gland was also common, with an incidence of 50.9% in the core study. This was similar to the 57% incidence in Harlan SD rats reported by Kaspareit and Rittinghausen (1999). Hyperplasia of the mammary gland occurred in 4.3% of the animals at 31 weeks and 10.7% of animals at 53 weeks. Hyperplasia was typically a diffuse lobular lesion, and lacked cellular atypia. Carcinomas of the mammary glands were also common in Harlan SD rats, with an incidence of 11.3% at 2 years. Adenomas were less frequent in Harlan SD rats, with an incidence of 3.0%. Adenomas were composed of glandular proliferations, and lacked the fibrous connective tissue component observed in fibroadenomas. There was no invasion and little cellular atypia in adenomas in contrast to the carcinomas of the mammary gland. The incidences of mammary gland tumors in Harlan SD females was greater than that of female Fischer rats for all tumor types; female Fischer rats had incidences of 44% for fibroadenomas, 2% for carcinomas, and 1% for adenomas (National Institute of Environmental Health Sciences, 2003).

Pancreas—Exocrine and Endocrine

Lesions of the pancreatic acinus seen at 2 years included atrophy (incidence 3.8%), hyperplasia (incidence 3.0%), and adenoma (incidence 0.3%). Sporadic occurrences of atrophy and hyperplasia were seen at the interim sacrifices. When atrophic, the amount of normal pancreatic acinar tissue was decreased and replaced by connective tissue, including adipose tissue. Frequently there was a mild infiltrate of mononuclear cells. Hyperplasia consisted of a focal area of enlargement due to increased numbers of cells of acinar origin; it was differentiated from the single adenoma based on size (with an adenoma having a diameter of >3 mm) similar to the criteria used by the NTP in Fischer rats (Boorman et al., 1990).

Six adenomas (incidence 1.6%) and 2 carcinomas (incidence 0.5%) of the pancreatic islets were observed in the core study. Islet cell adenomas were discrete masses whereas carcinomas were characterized by invasion and cellular atypia. The incidence of combined pancreatic islet cell adenoma and carcinoma in female Fischer rats is 1% (Haseman et al., 2003).

Pituitary Gland

Adenomas of the pars distalis were among the more common neoplasms observed in the female Harlan SD rats with an incidence of 41.2%. This incidence was similar to the incidence of 39% reported in Harlan SD rats by Kaspareit and Rittinghausen (1999), and is slightly higher than that seen in untreated female Fischer rats (incidence 34%) (Haseman et al., 2003). Hyperplasia of the pars distalis was also a common lesion, occurring in 34.1% of the animals.

Pars distalis adenomas were discrete lesions composed of a single cell type that caused compression of surrounding parenchyma. Cyst-like spaces that were filled with proteinaceous fluid or blood were often present within the adenomas. Hyperplasia was typically smaller, lacked the monotonous cell population, and lacked compression of the surrounding pituitary parenchyma. A single carcinoma of the pars distalis was observed (incidence 0.3%) in these studies. The distinguishing feature of the carcinoma was invasion into the brain. Hyperplasia and adenoma of the pars intermedia were much less common, with incidences of 0.8% and 1.1%, respectively.

Skin/Subcutaneous Tissue

Various neoplasms were recorded in the skin and subcutaneous tissue, but with the exception of fibroma (incidence of 1.4%) all of the neoplasms were single occurrences with an incidence of 0.3%. The neoplasms recorded included basal cell carcinoma, fibrosarcoma, sarcoma, malignant schwannoma, keratoacanthoma, squamous cell papilloma, and trichoepitehlioma. All of these tumors had incidences of less than 1% in control female Fischer rats (Boorman et al., 1990).

Thyroid Gland

Proliferative lesions of the thyroid C-cells were frequent. In the female Harlan SD core study, there was an incidence of 31.3% hyperplasia, 26.2% adenoma, and 3.8% carcinoma. The incidences reported in Harlan SD rats by Kaspareit and Rittinghausen are similar for neoplasms: 28% for adenomas and 1% for carcinomas; they did not report an incidence for hyperplasia (Kaspareit and Rittinghausen, 1999). Incidences of C-cell tumors were somewhat lower in untreated female Fischer rats; they had an incidence of 16% for adenomas and carcinomas combined (Haseman et al., 2003).

TABLE 3.—Incidences of common neoplastic lesions in control female Harlan Sprague–Dawley and Fischer rats.

Lesion	Incidence		
	Harlan Sprague–Dawley rats, gavage studies, NTP-2000 diet	Fischer rats, feed studies, NTP-2000 diet[1]	Fischer rats, gavage studies, NIH-07 diet[2]
Uterine polyp	16% (58/371)	17% (60/360)	20.2% (390/1934)
Mononuclear cell leukemia	<1% (2/371)	24% (87/360)	19.3% (377/1950)
Clitoral gland adenoma/carcinoma	<1% (1/363)	15% (52/353)	4.2% (83/1950)
Pituitary gland, pars distalis, adenoma or carcinoma	41% (153/369)	34% (121/359)	42.8% (813/1901)
Thyroid gland C-cell adenoma or carcinoma	30% (110/367)	16% (58/360)	11.4% (218/1913)
Mammary gland fibroadenoma	71% (263/371)	44% (160/360)	27.5% (536/1950)

[1]Haseman, J. K., Ney, E., Nyska, A., and Rao, G. N. (2003). Effect of diet and animal care/housing protocols on body weight, survival, tumor incidences, and nephropathy severity of F344 rats in chronic studies. *Toxicol Pathol* **31**, 674–81.

[2]Boorman, G. A., Eustis, S. L., Elwell, M. R., Montgomery, C. A. Jr., and MacKenzie, W. F. (eds.) (1990). *Pathology of the Fischer Rat*. Academic Press, Inc., New York.

C-cell hyperplasia consisted of focal or diffuse increases in the number of C-cells. As the cells were morphologically similar in hyperplasia and adenoma, size was usually the criterion used to differentiate focal hyperplasia and adenoma. Focal hyperplastic lesions were more common than diffuse lesions and were smaller than the area of 5 typical thyroid follicles. Large hyperplastic lesions and adenomas both may be associated with compression of surrounding follicles. Although C-cell carcinomas usually displayed minimal cellular atypia, differentiating carcinomas from very large adenomas was dependent on the presence of invasion through the capsule or into blood vessels by the carcinomas.

Thyroid gland follicular adenomas occurred with an incidence of 0.5%, similar to the 1% reported in Harlan SD rats by Kaspareit and Rittinghausen (1999). Thyroid gland follicular cell adenomas occurred in 0.9% of control female Fischer rats (Boorman et al., 1990).

Uterus

There were several lesions observed in the uterus in these studies, but with some notable exceptions, the incidences in controls were below 1%. The exceptions were squamous metaplasia (44.2% incidence), cystic endometrial hyperplasia (59.3% incidence), and stromal polyps (15.6% incidence).

Squamous metaplasia of the uterus is not a lesion commonly observed in NTP control Fischer rats (NTP database: ⟨http://ntp.niehs.nih.gov/ntpweb/index.cfm?objectid=72016020-BDB7-CEBA-F3E5A7965617C1C1⟩). In these Harlan SD rats, the normal epithelial surface of the uterus was often replaced by stratified squamous epithelium. Minimal-to-mild lesions only had a few cell layers of squamous epithelium, while severe lesions had a very thick layer of squamous epithelium covered by abundant keratin; these more severe lesions were grossly visible. The exact mechanism is not known; however, prolonged estrogen exposure has been associated with squamous metaplasia in rats for many years (Mceuen, 1936). In the case of Sprague–Dawley rats, females have difficulty ovulating as they age, due to an inadequate LH surge, and so the follicles remain in the ovary, secreting estrogen. The amount of time a female rat spends in estrus rises, as does the level of endogenous estrogen, as she ages. This may lead to the squamous metaplasia (Eldridgea et al., 1999).

Cystic endometrial hyperplasia in its mildest form consisted of slightly dilated glands lined by hyperplastic epithelial cells. In severe cases, there was a cystic dilatation of the glands, often grossly visible.

Stromal polyps were similar in incidence in Harlan SD rats (16%) and in Fischer rats (17%) (Haseman et al., 2003). Morphologically, they were similar to those observed in Fischer rats. They were attached to the surface endometrium by a stalk that was typically smaller in diameter than the largest part of the polyp. However, due to sectioning artifact, the attachment of the stalk of the polyp was not always present in the section, and the polyp may appear as an unconnected piece of endometrium in the lumen of the uterus. Smaller polyps could also be confused with the normal irregularities of the surface endometrium cut on cross-section. The polyp itself was usually composed of loose fibrovascular endometrial stroma with a few entrapped endometrial glands.

Other Tissues

Numerous other tissues had a low incidences of tumors recorded in core study animals; these included osteosarcoma in bone; lipoma in bone marrow; astrocytoma, oligodendroglioma, and medulloblastoma in the brain; neural crest tumor of the ear pinna; squamous cell carcinoma in the forestomach; leiomyoma and carcinoma in the large intestine; leiomyosarcoma and fibrosarcoma in the small intestine; malignant schwannoma in the mesentery; squamous cell carcinoma in the oral cavity; luteoma, malignant granulosa cell tumor and cystadenoma in the ovary; adenoma in the parathyroid gland; fibrous histiocytoma in skeletal muscle; thymoma in the thymus; fibrosarcoma and neurofibrosarcoma in the periodontal tissue; papilloma in the urinary bladder; sarcoma, squamous cell papilloma, squamous cell carcinoma and malignant schwannoma in the vagina; carcinoma of the Zymbal's gland, and mononuclear cell leukemia (large granular lymphocyte leukemia) and malignant lymphoma in multiple organs.

With the exception of squamous cell carcinomas of the oral cavity and malignant lymphoma, which both had incidences of 1.1%, all of the aforementioned neoplasms occurred at an incidence of 0.5% or less. The low incidence of mononuclear cell leukemia is in sharp contrast to the relatively high incidence of this lesion in untreated female Fischer rats (24%) (Haseman et al., 2003).

CONCLUSION

The overall survival rate in Harlan Sprague–Dawley rats, used as controls in 7 chronic (24-month) gavage studies, was 41.5%, with a mean life span of 628.7 days compared to a survival rate of 76% and a mean life span of 707 days for control female Fisher rats used in studies by the NTP

(Haseman et al., 2003). The maximum mean weekly body weight was 387.3 grams for Harlan SD and 320 grams for Fischer 344 rats.

The most commonly observed neoplasms in the control Harlan SD rats were mammary gland fibroadenoma (71%), tumors of the pars distalis of the pituitary (41%) and thyroid gland C-cell tumors (30%). Female Fischer rats had incidences of 44% for mammary gland fibroadenomas, 34% for tumors of the pars distalis, and 16% for thyroid gland C-cell tumors. Fischer rats had a 15% incidence of clitoral gland tumors while the Harlan SD rats had an incidence of <1% (Table 3).

In contrast to Fischer F344 rats, the Harlan SD rats had a high incidence of squamous metaplasia of the uterus (44.2%). This lesion was seen as early as 14 weeks. The Harlan SD rats had a very low incidence of mononuclear cell leukemia (0.5%). Mononuclear cell leukemia occurs at an incidence of 24% in Fischer rats, much greater than that seen in the Harlan SD. Mononuclear cell leukemia can cause significant secondary effects, especially in the liver.

Important uses of historical data, such as presented in this article, are the guidance they can provide investigators in selection of research models and the guidance in interpreting the subsequent results.

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