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The stability of historical control data for common neoplasms in laboratory rats: adrenal gland (medulla), mammary gland, liver, endocrine pancreas, and pituitary gland

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Abstract

Time-related changes in the incidences of spontaneous neoplasms in adrenals (medulla), mamma, liver, pituitary, and (endocrine) pancreas were assessed statistically in Wistar, Sprague–Dawley, and F344 rats employed by BASF, Germany and major European contract research organizations over the last 20 years. *Negative trends* (7 of 80 cases) were observed for pituitary pars distalis adenomas in Sprague–Dawley males and females, for pancreas islet cell adenomas in BASF Wistar males and females, for benign adrenal pheochromocytomas in Sprague–Dawley males, for malignant pheochromocytomas in F344 males, and for mammary gland fibroadenomas in BASF Wistar females. *Positive trends* (13 of 80 cases) were observed for benign pheochromocytomas, mammary gland adenocarcinomas, and pancreas islet cell carcinomas in HanWistar females, for malignant pheochromocytomas and islet cell carcinomas in BASF Wistar males, for benign pheochromocytomas and islet cell adenomas in F344 males, for mammary gland fibroadenomas in Sprague–Dawley females, and for benign hepatocellular tumors in HanWistar males and females, and in BASF Wistar and Sprague–Dawley females. In 60 of 80 cases there were no statistically significant trends. These results indicate that the majority of tumor types showed no time trends and that, in each rat strain, certain tumor types are susceptible to slight positive or negative time trends. Accordingly, the validity and use of historical control data should be based on an organ- and strain-specific statistical analysis of tumor incidence over time.

Keywords: Spontaneous tumors; Rat; Adrenals; Liver; Mammary gland; Pituitary; Pancreas

1. Introduction

The sensitivity of carcinogenicity tests is impaired due to the 'background noise' of common spontaneous neoplasms, which are unrelated to treatment. Historical data on the incidences of spontaneous neoplasms in control animals are supportively used in the assessment of carcinogenicity trials to avoid false positive results. The validity of indiscriminate use of such historical control data rests on the premiss that the susceptibility of the test animals to spontaneous tumor formation does not change over time. However, there is accumulating evidence that this may not always be the case. Haseman et al. (1989) described a virtually linear increase of spontaneous tumors in the hematopoietic system in control male Fischer F344 rats (NCI/NTP studies) from approximately 10% in 1971 to nearly 50% in 1981. Eiben and Bomhard (1999) reported a tendency to lower incidences of pituitary tumors and adrenal pheochromocytomas in control male (WISW SPF Cpb) Wistar rats in 70 2-year studies terminated between 1975 and 1994 at the Toxicology facility of Bayer AG, Wuppertal, Germany. In female (WISW SPF Cpb) Wistar rats, pituitary gland and mammary gland tumors showed a marked and highly significant increase. More recent data from the BASF facility in Ludwigshafen,

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Germany and major European contract research organizations (CROs) indicate an increase in the incidence of spontaneous liver tumors in Wistar and Sprague–Dawley rats over time (van Ravenzwaay and Tennekes, 2002). While an increasing tumor susceptibility over time may compromise the validity of the highest recorded incidence in control animals and enhance the risk of a *false positive result*, indiscriminate use of historical tumor incidences in cases of decreasing tumor susceptibility over time may introduce the risk of *false negative results*. Accordingly, there is a need to make an overall assessment of the validity of historical data for common spontaneous neoplasms.

This paper focuses on the assessment of time-related changes in the historical control incidence of common spontaneous tumors in adrenals (benign and malignant pheochromocytoma), mamma (fibroadenoma and adenocarcinoma), liver (hepatocellular adenoma and carcinoma), pituitary (adenoma of pars distalis), and pancreas (islet cell adenoma and carcinoma) in Wistar, Sprague–Dawley, and Fischer 344 (F344) rats employed by the BASF facility in Ludwigshafen, Germany and major European CROs over the last 10–20 years.

2. Characterisation of investigated tumor types

The morphological criteria (nomenclature) elaborated by RITA (1999), the Registry of Industrial Toxicology Animal-data, published by the WHO, were used to characterize the investigated tumor types. However, it should be noted that over the years (in which the studies were evaluated) some of the diagnostic criteria were adapted within the RITA nomenclature.

2.1. Adrenal glands, medulla (for diagnostic features, see IARC, 1994)

2.1.1. Pheochromocytoma, benign

Well-delineated mass of medullary cells, minimal to marked compression of surrounding parenchyma, altered architecture with cells arranged in large solid clusters or thick trabeculae, growth pattern may be variable, mild to marked alteration in size, shape, and staining qualities of affected cells.

2.1.2. Pheochromocytoma, malignant

Invasion of capsule and periadrenal soft tissue, metastasis.

2.2. Mammary gland (for diagnostic features, see IARC, 1993)

2.2.1. Fibroadenoma

A benign tumor derived from mammary gland epithelium and connective tissue. It contains epithelial structures resembling ducts and acini, surrounded by layers of proliferated fibrous tissue. It is a round or irregular mass generally greater than 5 mm in diameter, composed of glandular epithelium (ducts, ductules, and/or alveoli) and fibrous connective tissue. Alveoli have lobular pattern in smaller neoplasms. Ducts, some dilated and cystic, may be present. Epithelium generally is uniform and single-layered. Atypia or stratification, if present, is focal and does not constitute an expanding mass. Alveoli and ductules are surrounded by prominent mature, connective tissue stroma.

2.2.2. Adenocarcinoma

A malignant tumor arising from the glandular tissue. Typically it invades the surrounding or underlying tissues via the basement membrane and metastasizes through the lymphatic vessels. May arise from focal hyperplasia with atypia in ducts, ductules or alveoli; may also arise from foci of atypia in adenoma or fibroadenoma. When presenting, or associated with, adenoma or fibroadenoma, the malignant component must constitute an expanding mass that compresses and displaces the benign components. Consists of epithelium arranged in alveolar, ductular, papillary or solid structures or frequently combinations of these. The neoplastic epithelium exhibits cellular atypia, pleomorphism, or diffuse stratification with solid nests or lobules of cells. Cellular atypia consists of altered size or shape of cells, altered nuclear/cytoplasmic ratio, altered size, shape or chromatin content of nucleus, and altered staining quality of cytoplasm.

2.3. Pituitary gland (for diagnostic features, see IARC, 1994)

2.3.1. Adenoma of pars distalis

A benign tumor of the glandular cells of pars distalis. The tumor is well demarcated and shows compression of adjacent tissue in at least one quadrant of its margin, or it is a proliferative lesion larger than a half of pituitary lobe. The growth pattern may angiomatous, cystic, hemorrhagic, trabecular or solid.

2.4. Liver (for diagnostic features, see IARC, 1997)

2.4.1. Hepatocellular adenoma

A benign tumor derived from the hepatocytes. The tumor is sharply demarcated from the surrounding parenchyma which is compressed. Within the tumor the lobular hepatic architecture is lost. The cells are arranged in sheets or plates, the plates not thicker than one to two cells. The hepatic plates of the adenoma usually impinge on the surrounding liver plates at an angle. Cellular atypia may be seen as well as varying mixture of cellular change in the form of glycogen

deposition, cytoplasmic eosinophilia and basophilia, or formation of hyaline droplets.

2.4.2. Hepatocellular carcinoma

A malignant tumor derived from the hepatocytes. It shows locally invasive growth or formation of distant metastases. The tumor cells may exhibit a marked pleomorphism. According to the pattern of their arrangement the following tumor types can be recognized: *acinar* in which the neoplastic cells surround central clear spaces, *solid* formed by sheets of cells, and *trabecular* composed of irregular trabeculae formed in some areas by cell plates more than two cells in thickness.

2.5. Endocrine pancreas (for diagnostic features, see IARC, 1994)

2.5.1. Islet cell adenoma

A benign tumor derived from the cells forming islets of Langerhans. The tumor compresses surrounding tissue and is usually solitary and frequently encapsulated. The pale tumor cells are arranged in perivascular sheets, nests, and ribbons.

2.5.2. Islet cell carcinoma

The histological features are similar to those of islet cell adenoma, however, the tumor cells are polyhedral, fusiform or slightly pleomorphic. Local invasion of the tumor capsule and adjacent exocrine tissue as well as moderate mitotic activity and distant metastases (e.g., into the liver) are indicative for malignancy.

3. Investigated rat strains

3.1. Wistar rats

This rat strain is used both as a toxicology model and a general, all purpose, outbred model for use in biomedical research. Its body weight averages less than Sprague–Dawley rats yet it exhibits comparable reproductive performance. The original stock was obtained from the Wistar Institute, Philadelphia, PA. The following sub-strains were analyzed for time-related changes in the incidence of spontaneous tumors: the HanIbm:WIST rat, hereafter referred to as HanWistar rat, a cesarean derived stock from a Wistar colony maintained at the Hannover Institute in Germany (data on approximately 2500 control animals of each sex used in carcinogenicity trials from 1981 to 1998, provided by a CRO), and the Chbb:THOM (SPF) Wistar rat used by BASF (data on 1780 control animals of each sex used in carcinogenicity trials from 1981 to 1998). The data were divided into four time segments of 4–5 years. The differences in time segment length were a result of an uneven distribution of carcinogenicity trials over time.

3.2. Sprague–Dawley (SD) rats

This rat strain is a widely, general-purpose research model used in virtually all disciplines of biomedical research including pharmacology and toxicology. The strain originates from Sprague–Dawley and is maintained as an outbred colony. Data on the Crl:CD (SD) BR sub-strain, submitted by two different CROs that used the same animal breeder and the same animal diet, were analyzed (each laboratory provided data on approximately 1500 control animals of each sex used in carcinogenicity trials from 1986 to 1996). The data were divided into three time segments of 2–4 years. The differences in time segment length were a result of an uneven distribution of carcinogenicity trials over time.

3.3. Fischer 344 rats

This rat strain is commonly used for cancer research, toxicology and ageing studies. The Fischer 344 (F344) is the inbred model of choice for the U.S. National Toxicology Program (NTP) Carcinogen Bioassay Program and the U.S. National Institute on Ageing. The origin of the strain is as follows: to NIH in 1951 from Heston, to Heston in 1949 from Curtis, Columbia University Institute for Cancer Research. Data submitted by a CRO on approximately 1450 control animals of each sex used in carcinogenicity trials from 1986 to 1996 were divided into four time segments of 2–4 years. The differences in time segment length were a result of an uneven distribution of carcinogenicity trials over time.

4. Assessment of time-related changes in spontaneous tumor incidence

Time-related trends in the spontaneous tumor incidence were assessed by the Jonckheere–Terpstra test (Jonckheere, 1954). For the statistical analysis the tumor rate of each study was used. This was done, as the study is the most relevant unit to take the variability between studies into account and to consider the dependency of the results on the conditions within each study.

5. Results

The available data indicated that for the individual rat (sub-)strain, husbandry conditions were similar in the time periods reported. In addition, for the Wistar rat sub-strains growth and mortality rates in the evaluated studies were substantially similar over time. For the other rats strains these data were not provided.

5.1. HanWistar rat

The mean incidence of *pars distalis adenomas in the pituitary* showed a slight numerical increase over time in females (Table 1B), with virtually no variation in the highest recorded incidence per time segment. The trend was not significant in statistical terms.

Benign pheochromocytomas in the adrenal medulla showed a positive trend ($p < 0.05$) in females (Table 1B), the incidences ranging from 0 to 3% and 0 to 7% in 1981–1987 and 1988–1998, respectively.

Malignant pheochromocytomas in the adrenal medulla showed a slight numerical decrease over time in males (Table 1A), the incidences ranging from 0 to 9% and 0 to 3% in 1981–1987 and 1988–1998, respectively. The trend was not significant in statistical terms. *Benign hepatocellular tumors* showed a positive trend ($p < 0.05$) in both genders (Tables 1A and 1B). In 1981–1984, both genders of the HanWistar rat rarely developed spontaneous liver tumors: liver adenomas ranged from 0 to 3% in males and females; liver carcinomas were not observed. From 1985 onwards, the HanWistar rat was more susceptible to spontaneous liver adenoma formation, the incidences ranging from 0 to 6% and 0 to 10% in males and females, respectively, and hepatocellular carcinomas were occasionally observed in males and females, the incidences ranging from 0 to 2%.

Adenocarcinomas in the female mammary gland showed a positive trend ($p < 0.01$), the incidences ranging from 0 to 10% and 7 to 14% in 1981–1993 and 1994–1998, respectively (Table 1B).

Islet cell carcinomas in the pancreas showed a positive trend in females ($p < 0.05$); in 1981–1984, islet cell carcinomas were not observed, whereas from 1985 onwards, the incidences ranged from 0 to 4% (Table 1B).

Table 1A
Spontaneous tumor incidence in male HanWistar rats over time

Starting year	81–84	85–87	88–93	94–98	81–98
Adrenals: Benign Pheochromocytoma					
Mean (%)	2.9	3.8	4.0	3.5	3.6
Range (%)	0–6	0–8	0–9	2–5	0–9
N	580	925	670	375	2550
Adrenals: Malignant Pheochromocytoma					
Mean (%)	1.4	1.8	0.7	0.5	1.25
Range (%)	0–6	0–9	0–3	0–2	0–9
N	580	925	670	375	2550
Liver: Hepatocellular Adenoma					
Mean (%)	1.0	1.8	3.0	2.9	2.1*
Range (%)	0–3	0–6	1–6	1–5	0–6
N	581	909	640	410	2540
Liver: Hepatocellular Carcinoma					
Mean (%)	0	0.7	0.2	0.2	0.3
Range (%)	0	0–2	0–1	0–1	0–2
N	581	909	640	410	2540
Pituitary: Pars Distalis Adenoma					
Mean (%)	36.5	44.5	40.6	43.3	41.5
Range (%)	14–54	30–57	22–71	32–49	14–71
N	570	894	638	379	2481
Pancreas: Islet Cell Adenoma					
Mean (%)	5.2	6.0	4.4	4.6	5.2
Range (%)	0–14	2–9	0–9	0–7	0–14
N	463	898	637	370	2368
Pancreas: Islet Cell Carcinoma					
Mean (%)	1.3	1.2	3.5	1.4	1.9
Range (%)	0–5	0–4	0–10	0–2	0–10
N	463	898	637	370	2368

Jonckheere–Terpstra test.
* $p < 0.05$.

Table 1B
Spontaneous tumor incidence in female HanWistar rats over time

Starting year	81–84	85–87	88–93	94–98	81–98
Adrenals: Benign Pheochromocytoma					
Mean (%)	0.9	1.1	2.4	2.1	1.5*
Range (%)	0–3	0–3	0–7	1–3	0–7
N	579	922	668	379	2548
Adrenals: Malignant Pheochromocytoma					
Mean (%)	0.5	0.2	0.1	0.3	0.3
Range (%)	0–4	0–1	0–1	0–1	0–4
N	579	922	668	379	2548
Liver: Hepatocellular Adenoma					
Mean (%)	1.0	2.7	4.5	3.9	3.0*
Range (%)	0–3	0–9	0–10	1–10	0–10
N	580	904	619	406	2509
Liver: Hepatocellular Carcinoma					
Mean (%)	0	0.9	0.3	0.5	0.5
Range (%)	0	0–2	0–2	0–2	0–2
N	580	904	619	406	2509
Mammary Gland Fibroadenoma					
Mean (%)	25.3	35.0	28.7	22.8	29.2
Range (%)	6–43	12–60	15–42	13–29	6–60
N	576	901	638	391	2506
Mammary Gland: Adenocarcinoma					
Mean (%)	3.8	4.8	5.3	9.7	5.5**
Range (%)	0–10	0–10	0–10	7–14	0–14
N	576	901	638	391	2506
Pituitary: Pars Distalis Adenoma					
Mean (%)	62.5	67.0	65.6	75.6	66.9
Range (%)	46–79	37–83	48–78	64–83	37–83
N	573	902	640	389	2504
Pancreas: Islet Cell Adenoma					
Mean (%)	0.9	2.5	1.5	2.2	1.9
Range (%)	0–4	0–6	0–6	0–4	0–6
N	469	903	597	370	2339
Pancreas: Islet Cell Carcinoma					
Mean (%)	0	0.6	0.7	1.4	0.6*
Range (%)	0	0–4	0–4	0–4	0–4
N	469	903	597	370	2339

Jonckheere–Terpstra test.
* $p < 0.05$.
** $p < 0.01$.

5.2. BASF Wistar rat

Fibroadenomas in the female mammary gland showed a negative trend ($p < 0.05$), the incidences ranging from 12 to 36% and 0 to 20% in 1981–1984 and 1994–1998, respectively (Table 2B).

Benign pheochromocytomas in the adrenal medulla were numerically slightly decreased in females (Table 2B), the incidences ranging from 0 to 25% and 0 to 15% in 1981–1989 and 1990–1998, respectively. The trend was not significant in statistical terms. *Malignant pheochromocytomas in the adrenal medulla* showed a positive trend in males ($p < 0.01$), the incidences ranging from 0 to 4% and 0 to 8% in 1981–1989 and 1990–1998, respectively (Table 2A).

Malignant hepatocellular tumors showed a positive trend in males and females (Tables 2A and 2B), the incidences ranging from 0 to 8% and 0 to 2% in 1981–1989, and from 0 to 14% and 0 to 10% in 1990–1998, respectively. The trend was not significant in statistical terms.

Table 2A
Spontaneous tumor incidence in male BASF Wistar rats over time

Starting year	81–84	85–89	90–93	94–98	81–98
Adrenals: Benign Pheochromocytoma					
Mean (%)	20.2	24.0	19.2	17.1	20.2
Range (%)	0–38	0–35	12–45	10–30	0–45
N	450	420	530	380	1780
Adrenals: Malignant Pheochromocytoma					
Mean (%)	0.4	0.5	1.7	2.1	1.2**
Range (%)	0–4	0–2	0–8	0–5	0–8
N	450	420	530	380	1780
Liver: Hepatocellular Adenoma					
Mean (%)	6.0	8.1	6.2	9.2	7.2
Range (%)	0–26	0–30	0–18	0–22	0–30
N	450	420	530	380	1780
Liver: Hepatocellular Carcinoma					
Mean (%)	3.1	3.1	5.1	5.8	4.3
Range (%)	0–8	0–6	0–14	0–10	0–14
N	450	420	530	380	1780
Pituitary: Pars Distalis Adenoma					
Mean (%)	23.1	29.0	30.8	19.2	26.0
Range (%)	6–33	16–40	20–40	10–28	6–40
N	450	420	530	380	1780
Pancreas: Islet Cell Adenoma					
Mean (%)	4.2	3.3	2.1	2.4	3.0*
Range (%)	0–10	1–10	0–5	0–4	0–10
N	450	420	530	380	1780
Pancreas: Islet Cell Carcinoma					
Mean (%)	0.2	0	0.9	1.3	0.6*
Range (%)	0–2	0	0–5	0–5	0–5
N	450	420	530	380	1780

Jonckheere–Terpstra test.
* $p < 0.05$.
** $p < 0.01$.

The mean incidence of *benign hepatocellular tumors* showed a positive trend in females ($p < 0.05$), but there was no such trend in the highest recorded incidence per time segment (Table 2B).

Islet cell adenomas in the pancreas showed a negative trend in males and females ($p < 0.05$ for males; $p < 0.01$ for females), the incidence in males ranging from 0 to 10% and 0 to 5% in 1981–1989 and 1990–1998, respectively; in females, the incidences ranged from 0 to 5% in 1981–1993, but no such tumors were observed in 1994–1998 (Tables 2A and 2B).

Islet cell carcinomas in the pancreas showed a positive trend in males ($p < 0.05$), the incidences ranging from 0 to 2% and 0 to 5% in 1981–1989 and 1990–1998, respectively (Table 2A).

Table 2B
Spontaneous tumor incidence in female BASF Wistar rats over time

Starting year	81–84	85–89	90–93	94–98	81–98
Adrenals: Benign Pheochromocytoma					
Mean (%)	9.1	9.3	6.0	6.9	7.8
Range (%)	2–22	0–25	0–15	0–15	0–25
N	450	420	530	379	1779
Adrenals: Malignant Pheochromocytoma					
Mean (%)	0	0.2	0.2	0	0.1
Range (%)	0	0–1	0–5	0	0–5
N	450	420	530	379	1779
Liver: Hepatocellular Adenoma					
Mean (%)	3.6	2.9	3.2	5.0	3.6*
Range (%)	0–20	0–30	0–20	0–10	0–30
N	450	420	530	380	1780
Liver: Hepatocellular Carcinoma					
Mean (%)	0.4	0.5	1.5	1.1	0.9
Range (%)	0–2	0–2	0–5	0–10	0–10
N	450	420	530	380	1780
Mammary Gland: Fibroadenoma					
Mean (%)	20.4	16.9	14.9	12.9	16.3*
Range (%)	12–36	9–30	5–25	0–20	0–36
N	450	420	530	380	1780
Mammary Gland: Adenocarcinoma					
Mean (%)	10.4	6.9	10.9	6.8	9.0
Range (%)	4–24	0–18	0–25	0–14	0–25
N	450	420	530	380	1780
Pituitary: Pars Distalis Adenoma					
Mean (%)	64.9	77.1	74.3	70.5	71.8
Range (%)	36–88	46–92	56–90	56–86	36–92
N	450	420	530	380	1780
Pancreas: Islet Cell Adenoma					
Mean (%)	2.2	1.0	0.2	0	0.8**
Range (%)	0–4	0–5	0–5	0	0–5
N	450	420	530	380	1780
Pancreas: Islet Cell Carcinoma					
Mean (%)	0	0.5	0.2	0.3	0.2
Range (%)	0	0–2	0–2	0–2	0–2
N	450	420	530	380	1780

Jonckheere–Terpstra test.
* $p < 0.05$.
** $p < 0.01$.

5.3. F344 rat

Benign pheochromocytomas in the adrenal medulla showed a positive trend in males ($p < 0.05$), the incidences ranging from 0 to 16% and 6 to 22% in 1986–1989 and 1990–1996, respectively (Table 3A).

Malignant pheochromocytomas in the adrenal medulla showed a negative trend in males ($p < 0.01$), the incidences ranging from 2 to 7% and 0 to 4% in 1986–1987 and 1988–1996, respectively (Table 3A).

Islet cell adenomas in the pancreas showed a positive trend in males ($p < 0.05$), with virtually no variation in the highest recorded incidence per time segment (Table 3A). No statistically significant trends were observed in female F344 rats (Table 3B).

5.4. Sprague–Dawley rat

The data of two independent CROs (# 1 and # 2) were analyzed.

Fibroadenomas in the female mammary gland (Table 4B) showed a positive trend in both CROs ($p < 0.01$ in CRO # 1; $p < 0.05$ in CRO # 2). In CRO # 1, the incidences of fibroadenomas ranged from 32 to 54% in 1986–1987, and from 26 to 70% in 1988–1993. In CRO # 2, the incidences of fibroadenomas ranged from 39 to 47% in 1988–1989, and from 47 to 66% in 1990–1996.

A negative trend in males and females was observed for *pars distalis adenomas in the pituitary* in CRO # 1 ($p < 0.05$), with virtually no change in the highest recorded incidence per time segment (Tables 4A and 4B). In addition, there was a negative trend for *benign pheochromocytomas in the adrenals* (Table 4A) in males ($p < 0.05$) and a positive trend for *hepatocellular adenomas* (Table 4B) in females ($p < 0.05$) in CRO # 1, associated with concomitant changes in the highest recorded incidence per time segment.

Table 3A
Spontaneous tumor incidence in male F344 rats over time

Starting year	86–87	88–89	90–92	93–96	86–96
Adrenals: Benign Pheochromocytoma					
Mean (%)	7.5	5.1	13.1	11.5	8.7*
Range (%)	3–16	0–11	6–22	7–22	0–22
N	374	490	320	270	1454
Adrenals: Malignant Pheochromocytoma					
Mean (%)	4.8	1.6	1.3	0.4	2.1**
Range (%)	2–7	0–4	0–4	0–2	0–7
N	374	490	320	270	1454
Liver: Hepatocellular Adenoma					
Mean (%)	2.1	1.2	5.3	1.9	2.5
Range (%)	0–8	0–11	2–8	0–4	0–11
N	375	490	320	270	1455
Liver: Hepatocellular Carcinoma					
Mean (%)	0.3	0.2	0.3	0	0.2
Range (%)	0–2	0–2	0–2	0	0–2
N	375	490	320	270	1455
Pituitary: Pars Distalis Adenoma					
Mean (%)	28.9	19.2	29.8	24.9	25.1
Range (%)	18–40	2–38	17–47	13–36	2–47
N	367	490	319	269	1445
Pancreas: Islet Cell Adenoma					
Mean (%)	2.4	2.5	4.7	5.6	3.5*
Range (%)	0–7	0–6	4–6	4–8	0–8
N	372	487	320	270	1449
Pancreas: Islet Cell Carcinoma					
Mean (%)	1.6	2.5	1.6	0.7	1.7
Range (%)	0–4	0–8	0–6	0–2	0–8
N	372	487	320	270	1449

Jonckheere–Terpstra test.
* $p < 0.05$.
** $p < 0.01$.

Table 3B
Spontaneous tumor incidence in female F344 rats over time

Starting year	86–87	88–89	90–92	93–96	86–96
Adrenals: Benign Pheochromocytoma					
Mean (%)	1.6	1.4	2.2	0.4	1.4
Range (%)	0–4	0–4	0–7	0–2	0–7
N	375	490	320	270	1455
Adrenals: Malignant Pheochromocytoma					
Mean (%)	0.8	1.4	0.6	0	0.8
Range (%)	0–3	0–6	0–3	0	0–6
N	375	490	320	270	1455
Liver: Hepatocellular Adenoma					
Mean (%)	1.6	0.4	2.8	3.0	1.7
Range (%)	0–8	0–2	0–4	0–8	0–8
N	375	490	320	270	1455
Liver: Hepatocellular Carcinoma					
Mean (%)	0.3	0.2	0	0	0.1
Range (%)	0–2	0–2	0	0	0–2
N	375	490	320	270	1455
Mammary Gland: Fibroadenoma					
Mean (%)	16.3	10.8	13.1	13.1	13.1
Range (%)	6–26	2–20	5–20	5–20	2–26
N	375	490	320	260	1445
Mammary Gland: Adenocarcinoma					
Mean (%)	1.1	0.6	1.3	1.2	1.0
Range (%)	0–4	0–4	0–4	0–2	0–4
N	375	490	320	260	1445
Pituitary: Pars Distalis Adenoma					
Mean (%)	39.7	29.5	50.0	38.3	38.3
Range (%)	23–62	7–46	36–67	29–46	7–67
N	363	488	320	266	1437
Pancreas: Islet Cell Adenoma					
Mean (%)	0.8	0.4	0.3	1.1	0.6
Range (%)	0–2	0–2	0–2	0–2	0–2
N	375	490	318	268	1451
Pancreas: Islet Cell Carcinoma					
Mean (%)	0.5	0	0.3	0.4	0.3
Range (%)	0–4	0	0–2	0–2	0–4
N	375	490	318	268	1451

No statistically significant trends observed.

Table 4A
Spontaneous tumor incidence in male Sprague–Dawley rats over time

Laboratory	# 1				# 2			
Starting year	86–87	88–90	91–93	86–93	88–89	90–92	93–96	88–96
Adrenals: Benign Pheochromocytoma								
Mean (%)	17.6	7.2	8.0	10.4*	17.1	12.4	13.1	13.8
Range (%)	8–30	2–12	2–14	2–30	15–22	6–28	5–23	5–28
N	398	430	650	1478	350	490	626	1466
Adrenals: Malignant Pheochromocytoma								
Mean (%)	4.3	1.2	1.7	2.2	1.4	1.6	1.9	1.7
Range (%)	0–14	0–4	0–9	0–14	1–4	0–3	1–3	0–4
N	398	430	650	1478	350	490	626	1466
Liver: Hepatocellular Adenoma								
Mean (%)	2.0	0.9	3.7	2.4	1.4	2.2	1.4	1.7
Range (%)	0–4	0–4	0–10	0–10	0–4	1–4	0–3	0–4
N	400	430	648	1478	350	490	626	1466
Liver: Hepatocellular Carcinoma								
Mean (%)	1.0	1.6	2.6	1.9	0.3	0	0.2	0.1
Range (%)	0–4	0–6	0–7	0–7	0–1	0	0–1	0–1
N	400	430	648	1478	350	490	626	1466
Pituitary: Pars Distalis Adenoma								
Mean (%)	59.7	50.4	46.0	51.0*	58.5	50.4	50.5	52.4
Range (%)	46–69	35–70	31–62	31–70	54–66	38–62	42–59	38–66
N	397	427	643	1467	349	490	624	1463
Pancreas: Islet Cell Adenoma								
Mean (%)	8.3	3.7	4.5	5.4	7.8	7.2	6.1	6.9
Range (%)	4–16	0–10	0–12	0–16	4–13	4–11	4–8	4–13
N	400	428	550	1378	348	487	623	1458
Pancreas: Islet Cell Carcinoma								
Mean (%)	2.8	1.6	2.5	2.3	0.6	1.4	1.9	1.4
Range (%)	0–6	0–6	0–9	0–9	0–1	1–4	0–4	0–4
N	400	428	550	1378	348	487	623	1458

Jonckheere–Terpstra test.
* $p < 0.05$.

6. Conclusions

Historical data on the incidences of spontaneous neoplasms in control animals are supportively used in the assessment of carcinogenicity trials to avoid false positive results. The validity of indiscriminate use of such historical control data rests on the premiss that the susceptibility of the test animals to spontaneous tumor formation does not change significantly over time. This premiss was assessed by a statistical analysis of time-related changes in the historical control incidence of common spontaneous tumors in adrenals (benign and malignant pheochromocytoma), mamma (fibroadenoma and adenocarcinoma), liver (hepatocellular adenoma and carcinoma), pituitary (adenoma of pars distalis), and pancreas (islet cell adenoma and carcinoma) in Wistar, Sprague–Dawley, and Fischer 344 (F344) rats employed by the BASF facility in Ludwigshafen, Germany and major European contract research organizations (CROs) over the last 10–20 years.

The results of the survey indicate that each rat strain and gender appears to have a specific spontaneous tumor profile. The highest susceptibility to mammary fibroadenomas and adenocarcinomas was observed in female Sprague–Dawley rats. The highest susceptibility to pituitary pars distalis adenomas was observed in female BASF Wistar and Sprague–Dawley rats. Male BASF Wistar rats showed the highest susceptibility to hepatocellular tumors and adrenal pheochromocytomas. Male HanWistar and Sprague–Dawley rats showed the highest susceptibility to islet cell tumors in the endocrine pancreas. In all rat strains, males were more susceptible than females to spontaneous tumors in adrenal medulla, liver, and pancreas, while females were more susceptible than males to spontaneous pars distalis adenomas in the pituitary.

The results of this survey demonstrate a number of cases of changing susceptibility of laboratory rats to spontaneous tumor formation over time (Table 5). The time-related differences in average tumor incidence

Table 4B
Spontaneous tumor incidence in female Sprague–Dawley rats over time

Laboratory	# 1				# 2			
Starting year	86–87	88–90	91–93	86–93	88–89	90–92	93–96	88–96
Adrenals: Benign Pheochromocytoma								
Mean (%)	3.0	1.6	2.5	2.4	4.6	3.5	3.2	3.6
Range (%)	0–8	0–10	0–10	0–10	3–10	2–6	2–5	2–10
N	399	429	630	1458	348	489	629	1466
Adrenals: Malignant Pheochromocytoma								
Mean (%)	0.4	0.2	0.3	0.3	0.6	0	0.5	0.3
Range (%)	0–2	0–2	0–3	0–3	0–4	0	0–1	0–4
N	399	429	630	1458	348	489	629	1466
Liver: Hepatocellular Adenoma								
Mean (%)	0.5	0.2	2.1	1.1*	0.3	0.4	0.6	0.5
Range (%)	0–2	0–2	0–5	0–5	0–1	0–2	0–2	0–2
N	400	430	630	1460	347	490	630	1467
Liver: Hepatocellular Carcinoma								
Mean (%)	0	0	0	0	0	0	0	0
Range (%)	0	0	0	0	0	0	0	0
N	400	430	630	1460	347	490	630	1467
Mammary Gland: Fibroadenoma								
Mean (%)	44.3	49.5	61.4	53.2**	41.3	61.0	59.8	55.8*
Range (%)	32–54	26–70	50–69	26–70	39–47	57–65	47–66	39–66
N	400	430	630	1460	344	488	625	1457
Mammary Gland: Adenocarcinoma								
Mean (%)	7.0	10.5	10.8	9.7	9.3	12.3	12.3	11.6
Range (%)	4–12	4–18	2–20	2–20	6–14	7–18	10–16	6–18
N	400	430	630	1460	344	488	625	1457
Pituitary: Pars Distalis Adenoma								
Mean (%)	75.6	76.1	66.9	72.0*	74.0	72.8	72.5	73.0
Range (%)	68–82	67–86	50–80	50–86	65–83	69–77	68–80	65–83
N	398	426	624	1448	350	489	630	1469
Pancreas: Islet Cell Adenoma								
Mean (%)	3.3	1.9	1.7	2.2	4.0	2.9	1.3	2.5
Range (%)	0–10	0–4	0–4	0–10	0–5	2–4	0–7	0–7
N	397	428	536	1361	346	489	630	1465
Pancreas: Islet Cell Carcinoma								
Mean (%)	0.8	0.9	0.4	0.7	0.3	0.2	0.3	0.3
Range (%)	0–4	0–4	0–2	0–4	0–1	0–1	0–1	0–1
N	397	428	536	1361	346	489	630	1465

Jonckheere–Terpstra test.
* $p < 0.05$.
** $p < 0.01$.

(which were obtained by pooling data within time segments) were usually relatively small, and time-related trends marked as statistically significant may thus be regarded as minor in view of the high variability of incidences in individual studies.

A negative trend in the incidence of *pars distalis adenomas in the pituitary* was observed in male and female Sprague–Dawley rats in CRO # 1 (Table 5), suggesting decreasing tumor susceptibility over time. However, there was virtually no change in the highest recorded incidence per time segment.

Time-related changes in the incidence of *benign and/or malignant pheochromocytomas in the adrenal medulla* were detected in all rat strains (Table 5). Benign pheochromocytomas showed a positive trend in HanWistar females and in F344 males, but a negative trend in Sprague–Dawley males (in CRO # 1). Malignant pheochromocytomas showed a positive trend in BASF Wistar males but a negative trend in F344 males. The positive trend for malignant pheochromocytomas in BASF Wistar males may reflect a higher degree of malignancy of a fairly common tumor in this sex or, alternatively, subtle differences in diagnostic criteria. Similarly, in F344 males, the positive trend for benign pheochromocytomas and the negative trend for malignant pheochromocytomas suggest a lower degree of

Table 5
Summary of time trends in spontaneous tumor incidence in rat strains

	HanWistar		BASF Wistar		F344		SD (CRO # 1)		SD (CRO # 2)	
	M	F	M	F	M	F	M	F	M	F
Pituitary Adenoma	→	→	→	→	→	→	↓	↓	→	→
Pheochromocytoma (B)	→	↑	→	→	↑*	→	↓	→	→	→
Pheochromocytoma (M)	→	→	↑*	→	↓*	→	→	→	→	→
Hepatocell. Adenoma	↑	↑	→	↑	→	→	→	↑	→	→
Hepatocell. Carcinoma	→	→	→	→	→	→	→	→	→	→
Islet Cell Adenoma	→	→	↓*	↓	↑	→	→	→	→	→
Islet Cell Carcinoma	→	↑*	↑*	→	→	→	→	→	→	→
Mamma: Fibroadenoma	NA	→	NA	↓	NA	→	NA	↑	NA	↑
Mamma: Adenocarcinoma	NA	↑	NA	→	NA	→	NA	→	NA	→

→, no trend; ↑, positive trend; ↓, negative trend; *, trend not associated with changes in combined incidence of benign and malignant tumors; NA, not analyzed; SD, Sprague–Dawley; (B), benign; (M), malignant; M, males; F, females.

malignancy of these tumors or differences in diagnostic criteria (there was no time-related trend in the total incidence of benign or malignant pheochromocytomas). The changing incidences over time of benign pheochromocytomas in HanWistar females and Sprague–Dawley males appear to be genuine cases of changing susceptibility to pheochromocytoma formation over time, and were associated with time-related trends in the total incidence of benign or malignant pheochromocytomas. The reference ranges for benign pheochromocytomas in HanWistar females and Sprague–Dawley males were stable from 1990 to 1996 and 1988 to 1993, respectively.

Time-related changes in the incidence of *female mammary tumors* were detected in three rat strains (Table 5). *Adenocarcinomas in the female mammary gland* showed a positive trend in HanWistar rats, suggesting increasing tumor susceptibility over time. *Fibroadenomas in the female mammary gland* showed a negative trend in BASF Wistar rats and a positive trend in Sprague–Dawley rats (detected in two independent CROs), indicating decreasing and increasing tumor susceptibility over time, respectively. Only the 1994–1998 data appear valid as reference ranges for the incidence of mammary fibroadenomas in BASF Wistar females.

Time-related changes in the incidence of *benign and/or malignant islet cell tumors in the pancreas* were detected in three rat strains (Table 5). *Islet cell adenomas* showed a negative trend in BASF Wistar males and females and a positive trend in F344 males. *Islet cell carcinomas* showed a positive trend in HanWistar females and in BASF Wistar males. In BASF Wistar males, the negative trend for benign islet cell tumors and the positive trend for malignant islet cell tumors suggest a higher degree of malignancy of islet cell tumors or differences in diagnostic criteria, with little change being noted in the combined incidence of benign and malignant islet cells tumors, but the negative trend for benign islet cell tumors in BASF Wistar females could be a genuine case of decreasing tumor susceptibility over time (the combined incidence of benign and malignant islet cells tumors showed a clear negative trend). Only the 1994–1998 data may be valid as reference ranges for the incidence of islet cell tumors in BASF Wistar females. Similarly, in F344 males, the positive trend for islet cell adenomas was associated with a positive trend in the combined incidence of benign and malignant islet cell tumors. However, there was little variation in the highest recorded incidence of islet cell adenomas per time segment. In HanWistar females, the positive trend for malignant islet cell tumors was not associated with major changes in the combined incidence of benign and malignant islet cell tumors from 1985 to 1998 and reference ranges for malignant islet cell tumors were also stable from 1985 to 1998.

Time-related changes in the incidence of benign *hepatocellular tumors* were detected in three rat strains (Table 5). Benign hepatocellular tumors showed a positive trend in male and female HanWistar and female BASF Wistar rats. A positive trend for hepatocellular adenomas was also detected in Sprague–Dawley females (in CRO # 1). In all cases, the positive trends were associated with time-related increases in the combined incidence of benign and malignant hepatocellular tumors. The high susceptibility of BASF Wistar rats to spontaneous liver tumor formation has been shown to be associated with a high sensitivity to liver tumor induction by nongenotoxic liver carcinogens (van Ravenzwaay and Tennekes, 2002), and the positive time trends for liver tumors in HanWistar and Sprague–Dawley rats suggest that these strains may also become more sensitive to liver tumor induction by nongenotoxic liver carcinogens.

In conclusion, trends in the degree of tumor malignancy in the absence of trends in total (benign or malignant) tumor incidence (which could also reflect differences in diagnostic criteria) were apparent for

Table 6
Changing tumor susceptibility over time

Rat strain/sex	Increased	Unchanged	Decreased
HanWistar			
Males	1	6	0
Females	4	5	0
BASF Wistar			
Males	2	4	1
Females	1	6	2
F344			
Males	2	4	1
Females	0	9	0
Sprague–Dawley (CRO # 1)			
Males	0	5	2
Females	2	6	1
Sprague–Dawley (CRO # 2)			
Males	0	7	0
Females	1	8	0

pheochromocytomas in BASF Wistar males and F344 males, and for islet cell tumors in BASF Wistar males and HanWistar females.

Although all rat strains showed statistically significant trends over time in the spontaneous tumor incidence in one or more organs, stable tumor incidence ranges over time prevailed in 60 out of 80 examined cases (Table 6). In fact, the tumor data of female F344 rats (Table 3B) and male Sprague–Dawley rats from CRO # 2 (Table 4A) revealed no statistically significant trends over time in any organ. In these cases, there are no principal objections against indiscriminate use of the existing historical data, but a different approach must be adopted when signs of drift are observed. In the cases with statistically significant trends (20 of 80 cases), positive trends (13 of 20 cases) predominated over negative trends (7 of 20 cases).

It was considered beyond the scope of this survey to investigate potential causes of time-related trends. The available data on the Wistar rat sub-strains, however, do not indicate that factors such as husbandry conditions, growth, and mortality rates would account for the observed changes over time. For the Wistar rats used in the laboratories of BASF, it can additionally be added that the team of pathologists did not change significantly over the last 15 years. Therefore, it seems improbable that personnel changes in the individual laboratories could account for the tumor drift. It could be speculated that minor changes in the dietary components or changes during the breeding process may potentially have had an influence, however, there are no data to substantiate this speculation.

Negative trends could lead to an incorrect (*false negative*) conclusion (i.e., absence of tumorigenic potential) if the comprehensive historical database were to be used. Although the risk of a false negative conclusion appears to be rather low (7 of 80 cases), indiscriminative use of historical control data is clearly not acceptable with negative trends. Positive trends enhance the risk of a false positive conclusion (e.g., carcinogenic potential), which could be corrected, in principle, by future re-evaluations (i.e., using future control tumor incidences). Identification of positive trends is particularly important in cases of minimal apparently test substance-related increases in tumor incidence.

In conclusion, the results of this analysis indicate that indiscriminate use of historical control data is inappropriate. Likewise, restricted use of historical control data is counterproductive when time-related trends are absent. We propose that the use of historical control data should be based on organ- and strain-specific statistical analysis of tumor data over time.

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