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The stability of historical control data for common neoplasms in laboratory rats and the implications for carcinogenic risk assessment

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Abstract

Time-related changes in the incidences of spontaneous neoplasms in skin (fibroma and keratoacanthoma), thyroid (C-cell and follicular cell adenomas/carcinomas), uterus (stromal polyp), testes (Leydig cell tumor) and hemolymphoreticular system (mesenteric lymph node hemangioma and malignant granular lymphocytic leukemia) were assessed statistically in Wistar, Sprague–Dawley and F344 rats employed by the BASF, Germany and major European contract research organizations over the last 20 years. *Negative trends* (*5 out of 80 cases*) were observed for skin fibromas in F344 males, for follicular cell adenomas in Han Wistar females and in Sprague–Dawley males and females, and for follicular cell carcinomas in Sprague–Dawley males. *Positive trends* (*8 out of 80 cases*) were observed for skin keratoacanthomas in Han Wistar males, for C-cell adenomas in BASF Wistar males and females, for stromal polyps in Han Wistar and Sprague–Dawley females, and for mesenteric lymph node hemangiomas in Han Wistar and Sprague–Dawley males and in BASF Wistar females. In 67 out of 80 cases there were no statistically significant trends. Tumor drift was not common but occurred far more often in outbred rat strains (Wistar and Sprague–Dawley) than in the inbred rat strain (F344). This observation suggests that tumor predisposition is genetically determined, that tumor drift is primarily caused by genetic drift and that non-genotoxic carcinogens operate by facilitating the expression of tumor predisposition in target cells.

Keywords: Spontaneous tumors; Rat; Skin; Thyroid; Uterus; Testes; Lymph node; Leukemia

1. Introduction

The sensitivity of carcinogenicity tests is impaired due to the 'background noise' of common spontaneous neoplasms which are unrelated to treatment, and 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 now ample evidence that this is not always the case (Haseman et al., 1989; Eiben and Bomhard, 1999; van Ravenzwaay and Tennekes, 2002; Tennekes et al., 2004). 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

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may introduce the risk of *false negative results*. We have recently reported time-related changes in the incidences of spontaneous neoplasms in adrenals (medulla), mammary gland, liver, pituitary and (endocrine) pancreas in Wistar, Sprague–Dawley and F344 rats employed by BASF-Germany and major European contract research organizations over the last 10–20 years (Tennekes et al., 2004). *Negative trends* 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* were observed for benign pheochromocytomas, mammary gland adenocarcinomas and pancreas islet cell carcinomas in Han Wistar 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 Han Wistar males and females, and in BASF Wistar and Sprague–Dawley females.

The present paper completes the survey by an assessment of time-related changes in the historical control incidences of common spontaneous tumors in the *thyroid* (C-cell and follicular cell adenomas and carcinomas), *uterus* (stromal polyp), *testes* (Leydig cell tumor), *skin* (fibroma and keratoacanthoma), and the *hemolymphoreticular system* (mesenteric lymph node hemangioma and malignant large granular lymphocytic leukemia) in Wistar, Sprague–Dawley and F344 rats employed by BASF-Germany and major European contract research organizations over the last 10–20 years. An overall assessment of the stability of historical control data for all investigated common neoplasms in the various rat strains and its implications for carcinogenic risk assessment is made.

2. Characterization 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. Skin (for diagnostic features, see IARC, 1992, 1993)

2.1.1. Fibroma

A benign mesenchymal tumor formed by fibroblasts and fibrocytes.

2.1.2. Keratoacanthoma

A well-demarcated, benign epithelial tumor, composed of single or multiple cavities which invaginate from the skin surface; an epithelial pore providing a direct connection between cavity and surface is characteristic. The well-differentiated stratified squamous epithelium shows acanthosis and frequently a papillary proliferation into the lumen. There are distinct features of abortive hair follicle formation, and lamellated keratin material collects in the lumen.

2.2. Thyroid (for diagnostic features, see IARC, 1994)

2.2.1. C-cell adenoma

A benign tumor derived from the calcitonin-producing C-cells of the thyroid. The tumor is well circumscribed. Compression of the adjacent tissue may be present but is not a regular feature. Solid sheets of large, pale cells replace thyroid follicles; some follicles may be entrapped by the tumor. The tumor may be encapsulated.

2.2.2. C-cell carcinoma

A malignant tumor derived from the calcitonin-producing C-cells of the thyroid. The tumor consists of solid or irregular groups of pale, sometimes small or fusiform neoplastic cells replacing the thyroid follicles. Invasion of the thyroid capsule, vascular invasion, invasion of adjacent tissues, for example the parathyroid gland, or formation of distant metastases are characteristic features.

2.2.3. Follicular cell adenoma

A benign tumor of the thyroid follicular cells. The tumor is well-demarcated, compresses the adjacent tissue, and may be encapsulated. The tumor cells are cuboidal or columnar, hyperchromatic in comparison to surrounding tissue, arranged in single or multiple layers with piling up of follicular cells. The cystic type is characterized by dilated follicular structures (macrofollicular pattern), the papillary type by papillary structures, and the solid type appears solid because of its microfollicular pattern in which the lumina often are not recognizable.

2.2.4. Follicular cell carcinoma

A malignant tumor of the thyroid follicular cells, an obvious mass without well-demarcated boundaries, disorganized growth pattern in solid clusters or sheets, showing invasion of capsule, adjacent tissue, or formation of distant metastases.

2.3. Uterus (for diagnostic features, see IARC, 1997)

2.3.1. Stromal polyp

A pedunculate mass growing into the lumen, composed of stromal cells and covered by epithelium.

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

2.4.1. Leydig cell tumor (Adenoma, Leydig cell)

A benign tumor arising from the Leydig cells or the gonadal stromal cells. It is composed of uniform, polyhedral cells with abundant, eosinophilic, finely granulated cytoplasm. Lipid vacuolation is sometimes present. The cell nuclei are mostly round and centrally located. Less well differentiated, basophilic cells with scanty cytoplasm, or spindle-shaped cells can occur in larger tumors. There are cystic areas containing proteinatious fluid or blood. The tumor compresses seminiferous tubules and mostly is not encapsulated.

2.5. Hemolymphoreticular system (for diagnostic features, see IARC, 1992, 1993)

2.5.1. Hemangioma in the mesenteric lymph node (not analyzed in F344 strain)

A benign tumor of the blood vessels, containing blood-filled spaces lined by a single layer of prominent uniform endothelial cells without atypia. The tumor is rarely encapsulated and may compress the surrounding tissue.

2.5.2. Large Granular Lymphocytic Leukemia (''LGL,'' analyzed in F344 strain only)

This so-called ''Fischer rat leukemia,'' also known as mononuclear cell leukemia (''MCL,'' Stefanski et al., 1990), occurs mostly in the spleen and liver, is leukemic by definition and consists of cells with reddish cytoplasmic granules in May Gruenwald–Giemsa stain, exhibiting positivity for OX-8 antibody.

3. Rat strains and statistical trend test for changes in spontaneous tumor incidence over time

The data bases for the various rat (sub-)strains and the statistical trend test for the detection of significant time-related changes in the spontaneous tumor incidence have been described in detail in the previous paper (Tennekes et al., 2004). Time-related trends in the spontaneous tumor incidence were assessed by the Jonckheere–Terpstra test (Jonckheere, 1954), which takes the observed tumor rate in each study into account.

4. Results

4.1. Han Wistar rat

The incidence of *C-cell adenomas in the thyroid* appeared to be increasing over time in males (Table 1A), the incidences ranging from 0 to 12% in 1981–1984, and from 0% to 25% from 1985 onwards. The trend, however, was not significant in statistical terms.

Table 1A
Spontaneous tumor incidence in male Han Wistar rats over time

	Starting year				
	81–84	85–87	88–93	94–98	81–98
Thyroid, C-cell adenoma					
Mean (%)	6.0	10.9	11.2	13.0	10.3
Range (%)	0–12	1–24	0–25	5–19	0–25
N	570	960	687	407	2624
Thyroid, C-cell carcinoma					
Mean (%)	2.5	1.1	0.7	1.5	1.4
Range (%)	0–9	0–6	0–4	0–3	0–9
N	570	960	687	407	2624
Thyroid, follicular cell adenoma					
Mean (%)	4.9	2.9	2.6	2.0	3.1
Range (%)	0–14	0–7	0–6	0–6	0–14
N	570	960	687	407	2624
Thyroid, follicular cell carcinoma					
Mean (%)	1.2	0.6	0.9	0.5	0.8
Range (%)	0–6	0–3	0–4	0–1	0–6
N	570	960	687	407	2624
Testes, Leydig cell tumor					
Mean (%)	3.3	4.7	4.3	3.8	4.2
Range (%)	0–8	1–10	0–10	0–9	0–10
N	581	959	690	372	2602
Skin, fibroma					
Mean (%)	1.9	5.0	4.1	3.9	3.9
Range (%)	0–6	0–14	0–12	0–8	0–14
N	572	883	634	381	2470
Skin, keratoacanthoma					
Mean (%)	**0.9**	**4.8**	**3.6**	**5.0**	**3.6***
Range (%)	0–6	0–16	0–6	0–10	0–16
N	572	883	634	381	2470
Mesenteric lymph node, hemangioma					
Mean (%)	**4.4**	**10.7**	**7.9**	**13.4**	**8.9***
Range (%)	0–14	4–20	0–26	11–20	0–26
N	567	899	635	374	2475

Jonckhere–Terpstra test (two-sided): $^{*}p \leqslant 0.05$

Follicular cell adenomas in the thyroid showed a negative statistically significant trend ($p < 0.05$) in females (Table 1B). In females, the incidences ranged from 0 to 9% in 1981–1984 and from 0 to 1% in 1994–1998. The combined incidence of benign and malignant follicular cell tumors in females showed a significant negative trend ($p < 0.05$).

The incidence of *follicular cell carcinomas in the thyroid* appeared to decrease over time in males (Table 1A), the incidences ranging from 0 to 6% in 1981–1984 and from 0 to 1% in 1994–1998. The trend, however, was not significant in statistical terms.

Endometrial stromal polyps in the uterus showed a positive trend ($p < 0.01$), with virtually no change in the highest recorded incidence per time segment (Table 1B).

Table 1B
Spontaneous tumor incidence in female Han Wistar rats over time

	Starting year				
	81–84	85–87	88–93	94–98	81–98
Thyroid, C-cell adenoma					
Mean (%)	7.2	10.2	14.8	8.1	10.4
Range (%)	0–14	2–24	6–27	3–14	0–27
N	573	966	683	405	2627
Thyroid, C-cell carcinoma					
Mean (%)	1.0	2.0	0.9	0.7	1.3
Range (%)	0–4	0–11	0–4	0–1	0–11
N	573	966	683	405	2627
Thyroid, follicular cell adenoma					
Mean (%)	**2.8**	**1.7**	**1.8**	**0.2**	**1.7***
Range (%)	0–9	0–4	0–4	0–1	0–9
N	573	966	683	405	2627
Thyroid, follicular cell carcinoma					
Mean (%)	0.7	0.1	0.3	0.5	0.3
Range (%)	0–2	0–1	0–2	0–1	0–2
N	573	966	683	405	2627
Uterus, endometrial stromal polyp					
Mean (%)	**5.0**	**4.2**	**11.5**	**11.1**	**7.4****
Range (%)	0–22	0–13	6–19	7–20	0–22
N	578	801	637	387	2403
Skin, fibroma					
Mean (%)	1.2	1.0	1.2	0.5	1.0
Range (%)	0–4	0–2	0–3	0–2	0–4
N	573	882	600	374	2429
Skin, keratoacanthoma					
Mean (%)	0	0	0	0	0
Range (%)	0	0	0	0	0
N	573	882	600	374	2429
Mesenteric lymph node, hemangioma					
Mean (%)	2.2	2.5	4.0	3.8	3.0
Range (%)	0–8	0–6	0–10	0–6	0–10
N	555	890	632	371	2448

Jonckhere–Terpstra test (two-sided): $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$.

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

	Starting year				
	81–84	85–89	90–93	94–98	81–98
Thyroid: C-cell adenoma					
Mean (%)	**4.4**	**6.0**	**9.4**	**11.1**	**7.7****
Range (%)	0–8	0–16	0–15	2–20	0–20
N	450	420	530	380	1780
Thyroid: C-cell carcinoma					
Mean (%)	0	0.2	0.6	0.5	0.3
Range (%)	0	0–2	0–5	0–5	0–5
N	450	420	530	380	1780
Thyroid: follicular cell adenoma					
Mean (%)	1.1	1.0	0.9	0.8	1.0
Range (%)	0–2	0–5	0–6	0–5	0–6
N	450	420	530	380	1780
Thyroid: follicular cell carcinoma					
Mean (%)	1.3	0.5	0.2	0.5	0.6
Range (%)	0–12	0–2	0–2	0–2	0–12
N	450	420	530	380	1780
Testes: Leydig cell tumor					
Mean (%)	35.1	38.1	44.0	42.4	40.0
Range (%)	16–52	0–70	35–55	30–60	0–70
N	450	420	530	380	1780
Skin: fibroma					
Mean (%)	4.2	3.6	1.9	2.9	3.1
Range (%)	0–14	0–8	0–10	0–10	0–14
N	450	420	530	380	1780
Skin: keratoacanthoma					
Mean (%)	0.9	2.1	2.6	1.3	1.8
Range (%)	0–4	0–10	0–10	0–5	0–10
N	450	420	530	380	1780
Mesenteric lymph node: hemangioma					
Mean (%)	8.4	6.7	9.6	7.6	8.2
Range (%)	0–20	0–15	2–20	2–20	0–20
N	450	420	530	380	1780

Jonckhere–Terpstra test (two-sided): $^{**}p \leqslant 0.01$.

Skin keratoacanthomas showed a positive trend in males ($p < 0.05$), the incidences ranging from 0 to 6% in 1981–1984 and from 0 to 16% from 1985 onward (Table 1A).

Hemangiomas in the mesenteric lymph node showed a positive trend in males ($p < 0.05$) (Table 1A). In males, the incidences ranged from 0 to 14% in 1981–1984 and from 0 to 26% from 1985 onwards.

4.2. BASF Wistar rat

C-cell adenomas in the thyroid showed a positive trend ($p < 0.01$) in males and females (Tables 2A and 2B). The incidences of C-cell adenomas in males and females ranged from 0 to 8% and 0 to 12% in 1981–1984 and from 0 to 20% and 0 to 30%, respectively, from 1985 onwards. The combined incidence of benign and malignant C-cell tumors showed a significant positive trend ($p < 0.01$) in males and females.

Hemangiomas in the mesenteric lymph node showed a positive trend in females ($p < 0.05$), the incidences ranging from 0 to 5% in 1981–1993 and from 0 to 12% in 1994–1998 (Table 2B).

4.3. F344 Rat

Skin fibromas and *keratoacanthomas* showed a negative trend in males (Table 3A), the incidences ranging from 0 to 18% and 0 to 10% and from 0 to 10% and 0 to 4% in 1986–1989 and 1990–1996, respectively, but the trend was statistically significant for skin fibromas only ($p < 0.05$). No statistically significant trends were observed in female F344 rats (Table 3B).

4.4. Sprague–Dawley rat

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

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

	Starting year				
	81–84	85–89	90–93	94–98	81–98
Thyroid: C-cell adenoma					
Mean (%)	**4.7**	**7.1**	**15.3**	**12.9**	**10.2****
Range (%)	0–12	0–20	2–30	0–20	0–30
N	450	420	530	380	1780
Thyroid: C-cell carcinoma					
Mean (%)	0.7	0.7	0.8	1.3	0.8
Range (%)	0–4	0–2	0–5	0–5	0–5
N	450	420	530	380	1780
Thyroid: follicular cell adenoma					
Mean (%)	1.1	0.7	0.4	0.8	0.7
Range (%)	0–4	0–6	0–5	0–10	0–10
N	450	420	530	380	1780
Thyroid: follicular cell carcinoma					
Mean (%)	0.9	0.7	0.8	0.5	0.7
Range (%)	0–4	0–10	0–2	0–4	0–10
N	450	420	530	380	1780
Uterus: stromal or glandular polyp					
Mean (%)	5.1	3.3	7.7	7.1	5.9
Range (%)	0–12	0–10	0–20	0–20	0–20
N	450	420	530	380	1780
Skin: fibroma					
Mean (%)	0.9	0.2	0	0.5	0.4
Range (%)	0–6	0–2	0	0–5	0–6
N	450	420	530	380	1780
Skin: keratoacanthoma					
Mean (%)	0.2	0.2	0.2	0	0.2
Range (%)	0–2	0–2	0–2	0	0–2
N	450	420	530	380	1780
Mesenteric lymph node: hemangioma					
Mean (%)	**1.8**	**0.4**	**2.5**	**3.4**	**2.1***
Range (%)	0–4	0–4	0–5	0–12	0–12
N	450	420	530	380	1780

Jonckhere–Terpstra test (two-sided): $^{*}p \leqslant 0.05$; $^{**}p \leqslant 0.01$.

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

	Starting year				
	86–87	88–89	90–92	93–96	86–96
Thyroid: follicular cell adenoma					
Mean (%)	1.3	0.8	2.2	1.5	1.4
Range (%)	0–3	0–2	0–5	0–2	0–5
N	371	489	320	268	1448
Thyroid: follicular cell carcinoma					
Mean (%)	2.4	0.6	1.6	0.7	1.3
Range (%)	0–6	0–2	0–6	0–2	0–6
N	371	489	320	268	1448
Thyroid: C-cell adenoma					
Mean (%)	8.9	7.6	9.1	12.3	9.1
Range (%)	2–20	2–12	6–15	6–22	2–22
N	371	489	320	268	1448
Thyroid: C-cell carcinoma					
Mean (%)	5.4	2.7	6.6	2.2	4.1
Range (%)	2–9	0–4	0–10	0–6	0–10
N	371	489	320	268	1448
Skin: fibroma					
Mean (%)	**11.7**	**9.0**	**4.7**	**5.9**	**8.2***
Range (%)	6–18	0–18	0–8	2–10	0–18
N	375	490	320	270	1455
Skin: keratoacanthoma					
Mean (%)	3.5	4.7	2.5	2.6	3.5
Range (%)	0–8	2–10	0–4	0–4	0–10
N	375	490	320	270	1455
Testes: Leydig cell tumor					
Mean (%)	88.8	90.0	89.4	88.6	89.3
Range (%)	76–97	84–95	86–94	82–96	76–97
N	374	490	320	210	1394
Malignant large granular lymphocytic leukemia					
Mean (%)	39.2	29.8	33.8	46.7	36.2
Range (%)	24–68	20–38	16–55	36–58	16–68
N	375	490	320	270	1455

Jonckhere–Terpstra test (two-sided): $^{*}p \leqslant 0.05$.

In CRO #2 (Tables 4A and 4B), *follicular cell adenomas in the thyroid* showed a negative trend in males ($p < 0.01$) and females ($p < 0.05$), and *follicular cell carcinomas* also showed a negative trend in males ($p < 0.01$). The combined incidence of benign and malignant follicular cell tumors also showed a negative trend in males ($p < 0.01$) and females ($p < 0.05$). Similar decreases in follicular cell tumor incidences over time were observed in CRO # 1, but these were statistically not significant. The negative trends were usually associated with lower tumor incidence ranges in the most recently collected data.

Endometrial stromal polyps in the uterus showed a positive trend ($p < 0.05$) in CRO #1 (Table 4B), with no consistent changes in the highest recorded incidence per time segment.

Hemangiomas in the mesenteric lymph node showed a positive trend ($p < 0.01$) in males in CRO #1 (Table 4A), the tumor incidence ranging from 0 to 4% in 1991–1993, while these tumors were not detected in trials initiated in 1986–1987. In CRO #2 such changes was not observed, tumor incidences ranging from 0 to 3% over all time points.

5. Discussion

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 results presented in this paper on common spontaneous tumors in the thyroid (C-cell and follicular cell adenomas and carcinomas), uterus (stromal polyp), testes (Leydig cell tumor), skin (fibroma and keratoacanthoma), and hemolymphoreticular system (mesenteric lymph node hemangioma and malig-

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

	Starting year				
	86–87	88–89	90–92	93–96	86–96
Thyroid: follicular cell adenoma					
Mean (%)	1.3	0.6	1.3	1.1	1.0
Range (%)	0–4	0–3	0–2	0–4	0–4
N	374	490	320	270	1454
Thyroid: follicular cell carcinoma					
Mean (%)	0.8	0.4	0.6	0.4	0.6
Range (%)	0–4	0–2	0–3	0–2	0–4
N	374	490	320	270	1454
Thyroid: C-cell adenoma					
Mean (%)	4.8	5.1	7.2	8.1	6.1
Range (%)	0–10	0–12	2–16	5–13	0–16
N	374	490	320	270	1454
Thyroid: C-cell carcinoma					
Mean (%)	3.5	3.3	0.6	2.2	2.5
Range (%)	0–8	0–8	0–2	0–7	0–8
N	374	490	320	270	1454
Skin: fibroma					
Mean (%)	1.3	1.4	0	1.5	1.1
Range (%)	0–3	0–4	0	0–4	0–4
N	375	490	320	270	1455
Skin: keratoacanthoma					
Mean (%)	0.8	0.4	0.3	0.4	0.5
Range (%)	0–2	0–2	0–2	0–2	0–2
N	375	490	320	270	1455
Uterus: endometrial stromal polyp					
Mean (%)	13.9	9.6	15.7	21.9	14.0
Range (%)	10–28	0–18	12–20	18–30	0–30
N	375	490	319	210	1394
Malignant large granular lymphocytic leukemia					
Mean (%)	13.6	10.8	15.9	22.2	14.8
Range (%)	6–22	4–16	6–33	12–37	4–37
N	375	490	320	270	1455

No significant trends observed.

nant large granular lymphocytic leukemia), extend the existing evidence that the susceptibility of laboratory rats to spontaneous tumor formation may change in the course of time.

Time-related changes in the incidence of *skin tumors* were detected in two rat strains. A negative trend in the incidence of *fibromas* was detected in F344 males, suggesting decreasing tumor susceptibility over time; the historical reference ranges appeared stable from 1990 to 1996, i.e. for a period of 7 years. *Keratoacanthomas* showed a positive trend in Han Wistar males, suggesting increasing tumor susceptibility over time.

Time-related changes in the incidence of *thyroid tumors* were detected in three rat strains. C-cell *adenomas* showed a positive trend in BASF Wistar males and females, and negative trends were detected for *follicular cell tumors* in Han Wistar and Sprague–Dawley rats. Follicular cell adenomas showed a negative trend in Han Wistar females and Sprague–Dawley males and females (in CRO $\neq 2$), and follicular cell carcinomas showed a negative trend in Sprague–Dawley males (in CRO $\neq 2$). Thus, Han Wistar and Sprague–Dawley strains showed signs of decreasing susceptibility to follicular cell tumor formation over time. Time-related increases in the mean incidence of *stromal polyps in the uterus* were detected in Han Wistar and Sprague–Dawley rats, suggesting increasing tumor susceptibility over time. However, there were no consistent changes in the highest recorded incidence over time. Time-related changes in the incidence of *hemangiomas in the mesenteric lymph node* were detected in Han Wistar males, in BASF Wistar females, and in Sprague–Dawley males (in CRO #1), suggesting increasing tumor susceptibility over time.

There were no significant time-related changes in the incidence of *Leydig cell tumors in the testes* in any rat strain. *Malignant large granular lymphocytic leukemia* showed no significant time-related trend in F344 rats.

The overall results of this survey, part of which were already published in this journal (Tennekes et al., 2004), indicate that tumor drift (increasing or decreasing tumor susceptibility) was not common but occurred far more often in the outbred Wistar and Sprague–Dawley rat strains (Tables 5 and 6) than in the inbred rat strain (F344). Tumor drift in outbred rat strains was demonstrated in the

- *liver* (hepatocellular adenomas in Han Wistar males and females, and in BASF Wistar and Sprague–Dawley females),
- *skin* (keratoacanthomas in Han Wistar males),
- *hemolymphoreticular system* (mesenteric lymph node hemangiomas in Han Wistar and Sprague–Dawley males, and in BASF Wistar females),
- *mammary gland* (adenocarcinomas in Han Wistar females, fibroadenomas in BASF Wistar and Sprague–Dawley females),
- *pituitary* (pars distalis adenomas in Sprague–Dawley males and females),
- *thyroid* (C-cell adenomas in BASF males and females, follicular cell adenomas in Han Wistar females and in Sprague–Dawley males and females, follicular cell carcinomas in Sprague–Dawley males),
- *uterus* (endometrial stromal polyps in Han Wistar and Sprague–Dawley females),
- *pancreas* (islet cell adenomas in BASF Wistar females) and *adrenals* (benign pheochromocytomas in Sprague–Dawley males).

The positive trends for *adrenal* benign pheochromocytomas and *pancreas* islet cell carcinomas in Han Wistar females and for *adrenal* malignant pheochromocytomas and *pancreas* islet cell carcinomas as well as the

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
Thyroid: follicular cell adenoma								
Mean (%)	5.0	5.2	3.8	4.5	**6.2**	**5.6**	**1.8**	**4.1****
Range (%)	2–10	2–10	0–6	0–10	3–8	3–8	0–4	0–8
N	399	425	632	1456	340	468	605	1413
Thyroid: follicular cell carcinoma								
Mean (%)	2.8	1.4	0.9	1.6	**1.5**	**0**	**0**	**0.4****
Range (%)	0–6	0–6	0–3	0–6	0–4	0	0	0–4
N	399	425	632	1456	340	468	605	1413
Thyroid: C-cell adenoma								
Mean (%)	3.3	6.1	5.1	4.9	16.5	15.2	14.0	15.0
Range (%)	0–10	2–16	0–15	0–16	13–20	11–19	11–17	11–20
N	399	425	632	1456	340	468	605	1413
Thyroid: C-cell carcinoma								
Mean (%)	0.8	1.2	0.6	0.8	2.4	0.2	0.8	1.0
Range (%)	0–6	0–4	0–2	0–6	0–5	0–2	0–2	0–5
N	399	425	632	1456	340	468	605	1413
Skin: fibroma								
Mean (%)	9.3	5.8	9.8	8.5	7.5	11.2	6.7	10.7
Range (%)	4–14	0–9	2–19	0–19	7–10	10–28	2–16	2–28
N	400	430	650	1480	348	465	626	1439
Skin: keratoacanthoma								
Mean (%)	6.0	4.2	4.8	4.9	6.7	13.8	7.8	9.4
Range (%)	0–18	0–11	0–11	0–18	0–23	0–33	4–12	0–33
N	400	430	650	1480	348	465	626	1439
Testes: Leydig cell tumor								
Mean (%)	7.0	2.8	4.8	4.8	7.7	4.1	3.5	4.7
Range (%)	2–12	0–7	0–14	0–14	4–16	1–12	2–7	1–16
N	400	430	557	1387	350	490	626	1466
Mesenteric lymph node: hemangioma								
Mean (%)	**0**	**0.2**	**1.6**	**0.7****	1.4	1.2	1.1	1.2
Range (%)	0	0–2	0–4	0–4	0–2	0–3	0–2	0–3
N	398	429	558	1385	348	489	619	1456

Jonckhere–Terpstra test (two-sided): $^{**}p \leqslant 0.01$.

negative trend for *pancreas* islet cell adenomas in BASF Wistar males were not associated with significant trends in the combined incidence of benign and malignant tumors in respective organs, indicating that these trends may not reflect genuine tumor drift.

By contrast, tumor drift was rare in the inbred F344 strain: a decreasing incidence over time of skin fibromas in males was the only genuine case. The positive trends for *adrenal* benign pheochromocytomas and *pancreas* islet cell adenomas as well as the negative trend for *adrenal* malignant pheochromocytomas in F344 males were not associated with significant trends in the combined incidence of benign and malignant tumors in respective organs, indicating that all of these trends may not reflect genuine tumor drift (Table 7). No cases of tumor drift were seen in female F344 rats.

The results of this survey strongly suggest that *tumor drift is primarily caused by genetic drift*, i.e., by variation over time in the degree of *genetically determined tumor predisposition* in given populations; such genetic drift can be expected to be rare in an inbred rat strain, such as F344, but to be much more likely in an outbred rat strain, such as Wistar or Sprague–Dawley.

The inference that *carcinogenesis is genetically determined* has major implications for carcinogenic risk assessment. It follows that *genotoxicity* is a prerequisite for *genuine* carcinogenicity. Although numerous carcinogenicity studies with *non-genotoxic* substances have yielded positive results, these carcinogenic effects were almost invariably associated with evidence of significant tumor predisposition in the target cells. The frequently observed induction of mouse hepatocellular tumors by non-genotoxic substances is an excellent case in point. However, this effect is not species-specific; the high susceptibility of BASF Wistar rats to spontaneous hepatocellular tumors has been shown to be associated with

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
Thyroid: follicular cell adenoma								
Mean (%)	2.3	0.7	1.1	1.3	**3.0**	**0**	**0.3**	**0.8***
Range (%)	0–4	0–2	0–4	0–4	1–8	0	0–2	0–8
N	399	428	622	1449	338	483	622	1443
Thyroid: follicular cell carcinoma								
Mean (%)	2.0	0.2	0.3	0.8	0	0	0.2	0.1
Range (%)	0–10	0–2	0–2	0–10	0	0	0–1	0–1
N	399	428	622	1449	338	483	622	1443
Thyroid: C-cell adenoma								
Mean (%)	5.3	5.1	3.5	4.5	8.6	11.2	8.7	9.5
Range (%)	2–12	0–15	0–15	0–15	5–12	6–15	6–12	5–15
N	399	428	622	1449	338	483	622	1443
Thyroid: C-cell carcinoma								
Mean (%)	1.0	1.4	0.3	0.8	2.1	0.4	0.5	0.8
Range (%)	0–2	0–6	0–2	0–6	0–6	0–1	0–2	0–6
N	399	428	622	1449	338	483	622	1443
Skin: fibroma								
Mean (%)	5.5	2.3	4.3	4.0	2.0	3.3	2.2	2.5
Range (%)	0–12	0–4	0–8	0–12	1–4	1–6	0–6	0–6
N	400	429	630	1459	350	490	630	1470
Skin: keratoacanthoma								
Mean (%)	0.8	0.2	0.5	0.5	0.9	1.2	1.1	1.1
Range (%)	0–4	0–2	0–2	0–4	0–3	0–3	0–3	0–3
N	400	429	630	1459	350	490	630	1470
Uterus: endometrial stromal polyp								
Mean (%)	**3.3**	**4.2**	**6.1**	**4.6***	12.0	3.7	4.9	6.2
Range (%)	0–12	2–7	0–13	0–13	6–15	2–5	2–13	2–15
N	400	430	490	1320	350	490	630	1470
Mesenteric lymph node: hemangioma								
Mean (%)	0	0	0.2	0.1	0	0.2	0.3	0.2
Range (%)	0	0	0–2	0–2	0	0–1	0–1	0–1
N	397	429	536	1362	349	489	629	1467

Jonckhere–Terpstra test (two-sided): $^{*}p \leqslant 0.05$.

similarly high sensitivity to liver tumor induction by non-genotoxic compounds (van Ravenzwaay and Tennekes, 2002). By contrast, the data base of the US National Toxicology Program (NTP) Carcinogen Bioassay Program indicates that the F344 rat, which shows a much lower predisposition to hepatocellular tumors, was far less susceptible to non-genotoxic liver carcinogens than the sensitive B6C3F$_1$ mice (van Ravenzwaay and Tennekes, 2002): approximately 25 and 75% of the compounds tested unequivocally positive in F344 rats and B6C3F$_1$ mice, respectively, but, and perhaps even more importantly, nearly 60% produced unequivocal evidence of liver carcinogenicity in B6C3F$_1$ mice and no evidence of liver carcinogenicity in F344 rats. Thus, even though the use of an inbred strain may carry fewer risks of tumor drift and false positive or false negative results, the outcome of a carcinogenicity study with non-genotoxic carcinogens in all likelihood primarily depends on (the presence or absence of significant) tumor predisposition in the target cell(s).

There is additional evidence in the data base of the US NTP Carcinogen Bioassay Program to indicate that essentially non-genotoxic mechanisms can modulate *significant tumor predisposition* in rats and mice. Increased body weights at 52 weeks were associated with increased incidences of (most frequently occurring) pituitary gland and mammary gland neoplasms in F344 rats and liver tumors in B6C3F1 mice (Haseman et al., 1997; Haseman et al., 1998). A reanalysis of data from 218 two-year rodent carcinogenicity studies carried out by the NTP indicated that the most frequently occurring tumors showed chemically related decreases far more frequently than chance expectation (Haseman and Johnson, 1996). Many of these decreases, particularly those for pituitary and mammary gland tumors, adrenal pheochromocytoma and uterine polyps in

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

	Han Wistar		BASF Wistar		F344		SD (CRO # 1)		SD (CRO # 2)	
	M	F	M	F	M	F	M	F	M	F
Pituitary adenoma	→	→	→	→	→	→	↓	↓	→	→
A. Pheochromocytoma (B)	→	↑*	→	→	↑*	→	↓	→	→	→
A. Pheochromocytoma (M)	→	→	↑*	→	↓*	→	→	→	→	→
Benign/malignant combined		→	→		→		↓			
Hepatocellular adenoma	↑	↑	→	↑	→	→	→	↑	→	→
Hepatocellular carcinoma	→	→	→	→	→	→	→	→	→	→
Benign/malignant combined	↑	↑		↑				↑		
Pancreas islet cell adenoma	→	→	↓*	↓	↑*	→	→	→	→	→
Pancreas islet cell carcinoma	→	↑*	↑*	→	→	→	→	→	→	→
Benign/malignant combined		→	→	↓	→					
Mammary fibroadenoma	NA	→	NA	↓	NA	→	NA	↑	NA	↑
Mammary adenocarcinoma	NA	↑	NA	→	NA	→	NA	→	NA	→
Thyroid C-cell adenoma	→	→	↑	↑	→	→	→	→	→	→
Thyroid C-cell carcinoma	→	→	→	→	→	→	→	→	→	→
Benign/malignant combined			↑	↑						
T. Follicular cell adenoma	→	↓	→	→	→	→	→	→	↓	↓
T. Follicular cell carcinoma	→	→	→	→	→	→	→	→	↓	→
Benign/malignant combined		↓							↓	↓
Uterus stromal polyp	—	↑	—	→	—	→	-	↑	—	→
Testes Leydig cell tumor	→	—	→	—	→	—	→	—	→	—
Skin fibroma	→	→	→	→	↓	→	→	→	→	→
Skin keratoacanthoma	↑	→	→	→	→	→	→	→	→	→
MLN hemangioma	↑	→	→	↑	NA	NA	↑	→	→	→
Fischer Rat Leukemia	NA	NA	NA	NA	→	→	NA	NA	NA	NA

→: no significant trend; ↑: significant positive trend; ↓: significant negative trend; *: significant trend not associated with significant trend in combined incidence of benign and malignant tumors; NA: not analyzed; SD: Sprague–Dawley; (B): benign; (M): malignant; M: males; F: females; A: adrenals; T: thyroid; MLN: mesenteric lymph node. Trend analyses for common spontaneous tumors in pituitary, adrenals, liver, pancreas, and mammary gland were previously reported in this journal (Tennekes et al., 2004).

F344 rats and liver tumors in B6C3F1 mice, were associated with reduced body weights frequently observed in the dosed groups. The chemically related decreased incidences of leukemia in F344 rats appeared to be related to chemically related splenic toxicity. Three drugs that affect the neuroendocrine system (amphetamine, methylphenidate, and codeine) caused decreases in body weights and in the incidence of spontaneously occurring mammary gland neoplasms in female F344/N rats in 2-year carcinogenicity studies (Dunnick et al., 1996), but the decrease in mammary gland tumors seen in female F344/N rats could not be fully explained by body weight decreases relative to control animals, and it was postulated by the authors that because these pharmaceuticals are thought to affect the biologic system through interaction with membrane receptors, this interaction and/or subsequent cell signaling events may play a role in the observed decrease in spontaneously occurring mammary gland neoplasms in the female F344 rat. Recently, an increase in pituitary pars distalis adenoma incidence and a decrease in testicular Leydig cell tumor incidence have been noted in F344 rats, in 2 year NTP dermal and inhalation studies (Nyska et al., 1998), and it was proposed by the authors that stress, related to individual caging, particularly among males, may directly have impaired testosterone synthesis and produced Leydig cell atrophy leading to a feedback increase in the synthesis of luteinizing hormone by the anterior pituitary, followed by anterior pituitary cell functional hypertrophy, hyperplasia, and eventually neoplasia. The presence of Helicobacter hepaticus has been associated with an increased incidence of liver neoplasms in male B6C3F1 mice, and increases in cell proliferation rates and apoptosis were observed in the livers of male B6C3F1 mice with H. hepaticus-associated hepatitis (Hailey et al., 1998). The introduction in 1994 of the NTP-2000 diet for F344 rats by the NTP was found associated with a decreased incidence of pituitary gland tumors in both sexes and decreased incidences of adrenal pheochromocytoma in males (Haseman et al., 2003). The incidence and severity of nephropathy was also decreased in animals receiving the NTP-2000 diet, especially males, and the authors inferred that decreased incidences of adrenal pheochromocytoma were related to reduced nephropathy severity in male F344 rats and confirmed earlier evidence of

Table 6
Frequency of significant time trends in spontaneous tumor incidence in rat strains[a]

	Han Wistar	BASF Wistar	F344	SD (CRO # 1)	SD (CRO # 2)
Males					
Number of cases investigated	15	15	15	15	15
Number of cases with significant trends	3 (20%)	4 (27%)	4 (27%)	3 (20%)	2 (13%)
				5 (33%)	
Number of significant positive trends	3 (20%)	3 (20%)	2 (13%)	1 (7%)	0
				1 (7%)	
Number of significant negative trends	0	1 (7%)	2 (13%)	2 (13%)	2 (13%)
				4 (27%)	
Number of cases of genuine tumor drift	3	1	1	3	1
				4	
Females					
Number of cases investigated	17	17	17	17	17
Number of cases with significant trends	6 (35%)	5 (29%)	0	4 (24%)	2 (12%)
				5 (29%)	
Number of significant positive trends	5 (29%)	3 (18%)	0	3 (18%)	1 (6%)
				3 (18%)	
Number of significant negative trends	1 (6%)	2 (12%)	0	1 (6%)	1 (6%)
				2 (12%)	
Number of cases of genuine tumor drift	4	5	0	4	2
				5	
Males and females					
Number of cases investigated	32	32	32	32	32
Number of cases with significant trends	9 (28%)	9 (28%)	4 (13%)	7 (22%)	4 (13%)
				10 (31%)	
Number of significant positive trends	8 (25%)	6 (19%)	2 (6%)	4 (13%)	1 (3%)
				4 (13%)	
Number of significant negative trends	1 (3%)	3 (9%)	2 (6%)	3 (9%)	3 (9%)
				6 (19%)	
Number of cases of genuine tumor drift	7	6	1	7	3
				9	

[a] Includes previously reported trend analyses for common spontaneous tumors in pituitary, adrenals, liver, pancreas, and mammary gland (Tennekes et al., 2004).

a positive correlation between the severity of chronic progressive glomerulonephropathy and the incidence of adrenal pheochromocytoma in male F344 rats at the NTP (Nyska et al., 1999).

Since non-genotoxic carcinogens also appear to operate by modulating the expression of significant tumor predisposition in target cells (in the species and strains of laboratory animals used), the reliability of the carcinogenicity bioassay for testing of non-genotoxic substances and the extrapolation of positive results to humans may be questioned. As inferred earlier, the likelihood of a positive result with non-genotoxic substances may well be determined by the degree of tumor predisposition of target cells in the test population. At the same time, non-genotoxic carcinogen may go undetected in cases of very high spontaneous tumor incidences (e.g. Leydig cell tumors in F344 rats with incidences approaching 100%). If carcinogenic effects are detected in the presence of significant tumor predisposition in target cells, such as in mouse or BASF Wistar rat hepatocytes, the question arises as to whether this effect can be regarded as relevant to man.

In the current European Union legislation (Commission Directive 93/21/EEC, 1993), the criteria used to conclude that an animal carcinogen is not relevant for humans state that

- "a substance should not be classified in any of the categories if the mechanism of experimental tumour formation is clearly identified, with good evidence that this process cannot be extrapolated to man,"
- "if the only available tumour data are liver tumours in certain sensitive strains of mice, without any further evidence, the substance may not be classified in any of the categories,"
- "particular attention should be paid to cases where the only available tumour data are the occurrence of neoplasms at sites and in strains where they are well known to occur spontaneously with a high incidence."

The present evidence indicates that a substance should not be classified in any of the (carcinogenic risk) categories if the only available tumor data are the occurrence of neoplasms at sites and in species

Table 7
Significant time-related trends in spontaneous tumor incidence in F344 rats

	Starting year				
	86–87	88–89	90–92	93–96	86–96
Adrenals: benign pheochromocytoma (males)					
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 (males)					
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
Adrenals: benign or malignant pheochromocytoma (males)					
Mean (%)	12.3	6.7	14.4	11.9	10.8
Range (%)	6–22	0–15	6–22	7–22	0–22
N	374	490	320	270	1454
Pancreas: islet cell adenoma (males)					
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 (males)					
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
Pancreas: islet cell adenoma or carcinoma (males)					
Mean (%)	4.0	4.9	6.3	6.3	5.2
Range (%)	0–8	0–14	4–10	4–10	0–14
N	372	487	320	270	1449
Skin: fibroma (males)					
Mean (%)	**11.7**	**9.0**	**4.7**	**5.9**	**8.2***
Range (%)	6–18	0–18	0–8	2–10	0–18
N	375	490	320	270	1455

Jonckheere–Terpstra test: $^{*}p < 0.05$; $^{**}p < 0.01$.

and strains where they are well known to occur spontaneously with a high incidence, with good evidence that this sensitivity cannot be extrapolated to man and the mechanism of experimental tumor formation does not involve genotoxicity and shows a clear no observed effect level.

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