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Frequency and time trends of spontaneous tumors found in B6C3F$_1$ mice oncogenicity studies over 10 years

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With 18 figures and 5 tables

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Key words: B6C3F$_1$-mice, mortality, tumor incidences, long-term studies, historical data carcinogenicity.

Summary

Historical data from 2883 B6C3F$_1$ mice used as controls in 29 two-year oncogenicity studies terminated between 1988 and 1998 were analyzed for possible time trends in mortality, terminal body weight and tumor incidences. There was no time trend in terminal body weights. Concerning mortality data a slight decreasing trend ($p > 0.05$) was evident in males, whereas in females mortality rates increased significantly ($p = 0.0009$). The overall tumor spectrum of the collectives used was roughly in line with the tumor profile known for B6C3F$_1$ mice. Most tumor types occurred in the hematopoietic tissue, liver, lungs, Harderian glands, vascular system, endocrinium (pituitary, adrenals and thyroids) or female reproductive organs. In comparison to literature data mice used in our lab exhibited less hepatocellular tumors and lung adenomas. Hepatocellular adenomas (females only) and carcinomas (both sexes) as well as adenomas in the Harderian glands decreased significantly over the time examined. For ovarian cystadenomas as well as uterine polyps and uterine stromal sarcomas significantly positive time trends were calculated. A positive time trend was also found for adrenal adenomas in males ($p < 0.05$) and histiocytic sarcomas in females ($p > 0.05$). Lymphomas occurred with increasing incidences over time in males ($p < 0.05$) and females ($p < 0.05$). Other factors such as genetic drifts might be responsible for these trends rather than changes in the body weights, which remained stable over 10 years.

Introduction

For the evaluation of the results of rodent oncogenicity studies rather precise knowledge on historical tumor data and positive or negative trends of spontaneous tumors of the animal strain used is necessary. B6C3F$_1$-mice are used widely for long-term studies and some publications reporting historical tumor data are available (HASEMAN et al. 1985; TAMANSO et al. 1988; CHANDRA and FRITH 1992 and HASEMAN et al. 1998; RAO et al. 1990b). Only one of this publication (RAO et al. 1990b) investigated possible trends in the tumor spectrum occurring over a longer period, however, these data were derived from different labs. In this paper interlaboratory variability are discussed as the major factor influencing time trends in tumor rates. Since the knowledge of possible trends in the mortality data and frequency of certain tumor types in the animal stock used is of interest and helpful in study evaluation, longitudinal analyses of this data were performed for 29 control groups used between 1988 and 1998. One special advantage of this data set is the fact that many important general conditions had remained almost unchanged for all studies within that period thus providing a well basis for comparison. Moreover, this paper intended to show whether mice used at Bayer AG show any correlation between increasing body weight and ascending tumor formation as reported for liver tumors by SEILKOP 1995 and HASEMAN et al. 1997. As earlier reported for Wistar rats (EIBEN and BOMHARD 1999) there is not a general rule for rodents that increasing or decreasing tumor rates must be correlated with changes in body weights, which was reported in many papers for Sprague Dawley and Fischer 344 rats (NOHYNEK et al. 1993 and RAO et al. 1990a).

Material and methods

Experimental animals, housing conditions and nutrition of the animals

All B6C3F$_1$ mice used were SPF bred and supplied by Bomholtgard Breeding and Research Center Ltd., 8680 Ry, Denmark. The mice were housed individually, under conventional conditions, in Makrolon® type II cages (SPIEGEL and GÖNNERT 1961) on low-dust, soft-wood shavings supplied by Ssniff GmbH, Soest. The room temperature was adjusted to 22 ± 2 °C. The humidity was set up to 55 ± 10%. An artificial light/dark cycle of 12 hours was used. From July 1991 onwards mice were kept in animal rooms located within a barrier system. Animal rooms and all cage furniture were cleaned weekly. Racks for cages were cleaned every four week. The diet consisted of certified Altromin® 1321 meal to which 1% peanut oil was added to avoid dust formation or Altromin® 1321 in pellet form (Altromin® 1324 in 2 studies only). In any case animals were fed ad libitum. Body weights were measured weekly (first 6 months) or bi-weekly thereafter. Food and tap water were given to the animals ad libitum.

Necropsy and histological technique

Mice dying intercurrently or killed by exsanguination in deep anesthesia (under diethyl ether narcosis) in moribund conditions were necropsied as soon as possible and subjected to gross pathological evaluation. Selected organs and all neoplasms deemed evaluable were fixed in buffered formaldehyde solution.

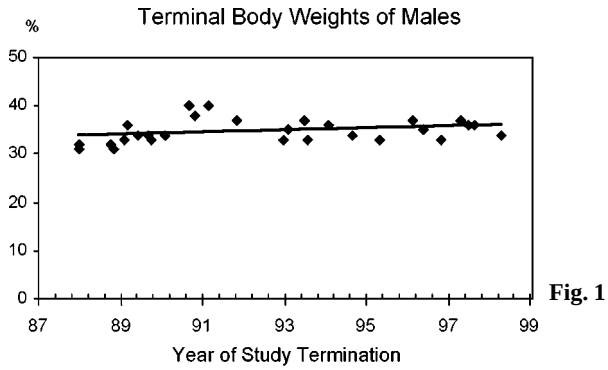


Fig. 1

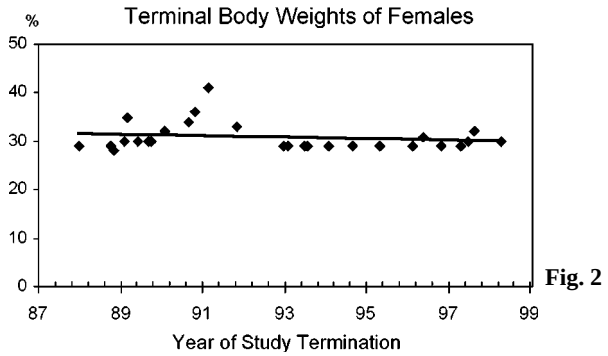


Fig. 2

Fig. 1–2. Results of the longitudinal analysis of terminal body weight data over 10 years. Data were received from B6C3F$_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) of body weight were calculated for each collective and plotted against the time point when the study was terminated.

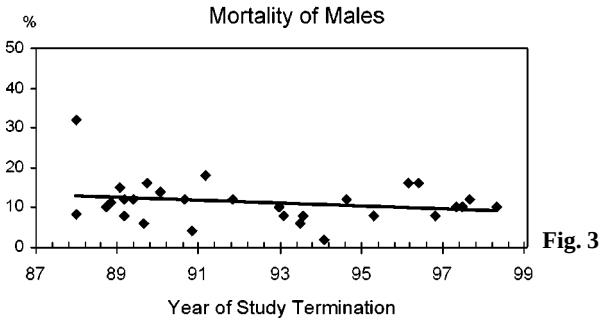


Fig. 3

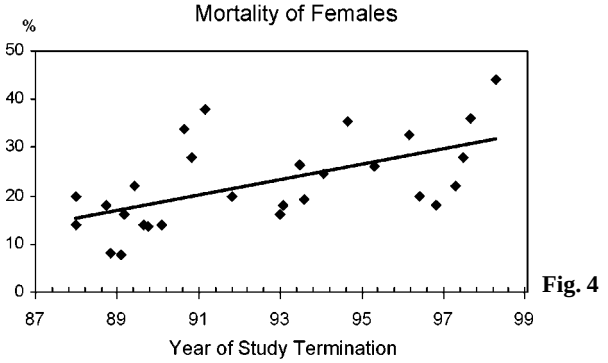


Fig. 4

Fig. 3–4. Results of the longitudinal analysis of mortality data over 10 years. Data were received from B6C3F$_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) of mortality were calculated for each collective and plotted against the time point when the study was terminated.

Table 1. Body weights and mortality in B6C3F$_1$ mice taken from 2-year studies.

	Males (n = 1471)		Females (n = 1452)	
	Mean	Min.–Max.	Mean	Min.–Max.
Terminal Body Weights (g)	34.8	31–40	30.6	29–41
Terminal Mortality (%)	11.2	2–32	22.4	8–44

All mice alive at termination after a study period of 24 months were anesthetized with diethyl ether, killed by exsanguination and investigated macroscopically. Organs as recommended by the guidelines for oncogenicity studies being in force were fixed as described above. The fixed organs and tissues were embedded in paraplast, cut into sections of about 5 microns thick and stained with hematoxylin and eosin. The bones were decalcified using EDTA. In some cases special fixatives and/or staining methods were used, usually to clarify tumor diagnosis.

The tumor nomenclature employed for tabulation relies as far as possible on proposals by MOHR et al. (2001).

Since the histological slides were not reviewed for the purpose of this paper, tumor subtyping of the original diagnosis made by the study pathologist could not be performed in several cases. Therefore, tumors of the pars distalis or of the pars intermedia had to be summerized as "Tumors NOS" of the pituitary gland in the summary tables. The same holds true for cortical neoplasias of the adrenal glands. Here, subcapsular cell tumors (A- or B-cell type) had to be combined with cortical lesions to the term "Tumor NOS".

Data and evaluation method

The data evaluated originate from 29 male and female groups (50 to 60 animals each) used as control groups in oncogenicity studies started at Bayer AG Toxicology in the years between 1986 and 1996. For each control collective cumulative mortality rate, terminal body weight as well as incidences (as rounded percentages) of frequent tumors (mean frequency >0.1%), overall tumors, and tumor-bearing animals were calculated. This data was the basis for mean value calculations (tables 1–4), which were then used for a longitudinal analysis done by plotting means against the time point the study was terminated. In cases where a remarkable positive or negative trend was evident in males and/or females a diagram showing the regression line calculated over the whole 10-year period by the EXCEL 5.0 program is given (see fig. 1–18). In order to make visible positive or negative time trends each regression line was evaluated statistically using the Students t-test by using SAS 6.12 on a Windows NT 4.0 Workstation platform (WEISBERG 1980). Multiple test corrections were not taken into account.

Tumor types with a frequency below 0.1% are not described in detail, but taken into account when incidences of overall tumors and of tumor-bearing animals were evaluated. Only primary tumors were taken into account.

Animals or organs that could not be investigated histopathologically due to autolysis were not considered in the evaluation. Tumors of the same type occurring in paired organs as well as several tumors of the same type in one organ were considered only once in the evaluation.

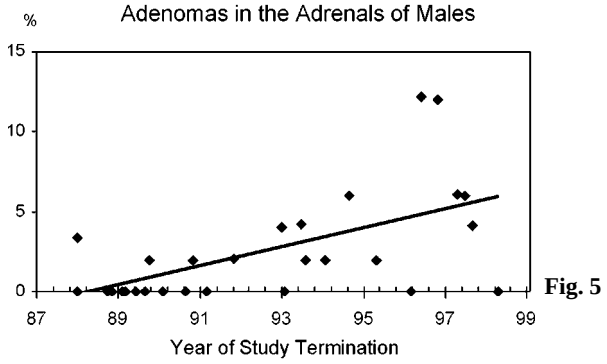


Fig. 5

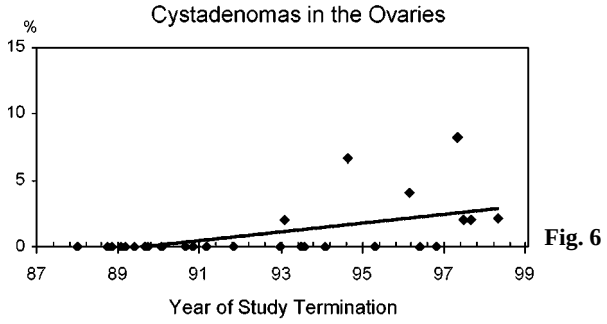


Fig. 6

Fig. 5–6. Results of the longitudinal analysis of adrenal adenomas and ovarian cystadenomas over 10 years. Data were received from $B6C3F_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each collective and plotted against the time point when the study was terminated.

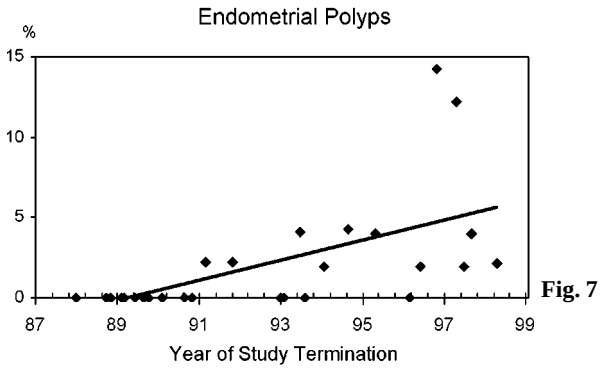


Fig. 7

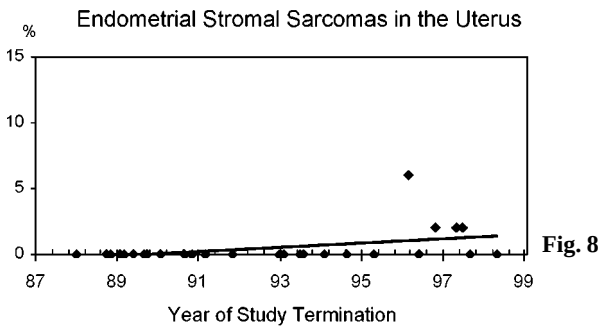


Fig. 8

Fig. 7–8. Results of the longitudinal analysis of uterine polyps and endometrial stromal sarcomas over 10 years. Data were received from $B6C3F_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each collective and plotted against the time point when the study was terminated.

Table 2. Frequency of tumors# in B6C3F$_1$ mice taken from 2-year studies: endocrine and reproductive organs.

Sex **Organ** / Tumor Type[1)]		**Percentage Incidence**			
		Males		**Females**	
		Mean	Min.-Max.	**Mean**	Min.-Max.
Pituitary					
– Adenoma, NOS	*b*[&)]	0.8	0–4.2	10.6	2.1–24.0
Adrenal Glands					
– Cortical Adenoma, NOS[$]	*b*	2.4[*)]	0–12.2(+)*	0.2	0–2.0
– Medullary Tumor	*b*	0.3	0–4.1	0.6	0–4.2
– Cortical adenocarcinoma	*m*[&)]	0.1	0–2.0	(0.1	
Thyroid Glands					
– Adenoma (follicular cells)	*b*	0.4	0–2.0	1.6	0–6.1
Parathyroid Glands					
– Adenoma	*b*	0		0.2	0–4.1
Pancreas					
– Acinar Cell Adenoma	*b*	0.1	0–2.0	0	
– Islet Cell Adenoma	*b*	0.3	0.2.0	0.6	0–4.0
Skin/Mammary Glands					
– Adenocarcinoma	*m*	–	–	0.5	0–2.2
Ovaries					
– Cystadenoma	*b*	–	–	0.9[*)]	0–8.2(+)*
– Tubulostromal Adenoma	*b*	–	–	0.3	0–2.1
– Adenoma, NOS	*b*	–	–	0.4	0–2.1
– Granulosa/Theca Cell Tumor	*b*	–	–	0.6	0–7.7
– Luteoma	*b*	–	–	0.6	0–4.2
– Teratoma	*b*	–	–	0.3	0–2.2
Uterus					
– Stromal Polyp	*b*	–	–	1.9[*)]	0–14.3(+)*
– Adenoma	*b*	–	–	0.5	0–6.0
– Fibroma	*b*	–	–	0.1	0–4.0
– Leiomyoma	*b*	–	–	0.8	0–6.1
– Leiomyosarcoma	*m*	–	–	1.0	0–4.0
– Sarcoma, NOS	*m*	–	–	0.4	0–2.0
– Endometrial Stromal Sarcoma Cell Tumor	*m*	–	–	0.4[*)]	0–6.0(+)*
– Adenocarcinoma	*m*	–	–	1.0	0–4.0
Vagina					
– Squamous Cell Carcinoma	*m*	–	–	1.0	0–2.1
Testes					
– Leydig Cell Adenoma	*b*	0.5	0–4.1	–	–

[#)] occurring with a mean frequency > 0.1%; [*)] frequency of this tumor type evaluated by a longitudinal analysis (see Figures); [&)] *b = benign m = malignant;* [1)] tumors found in paired organs were counted as one tumor; *significant trend $p \leq 0.05$; (–) decreasing trend over 10 years; (+) increasing trend over 10 years; $ includes subcapsular cell adenoma

Results

Mortality, body weight and tumor data of 1446 male and 1437 female B6C3F$_1$ mice taken from control groups were evaluated. All mean data including their variation range mentioned in this chapter is summarized in the tables 1–4.

Body weights and mortality

Terminal body weights: The mean terminal body weights of male and female mice remained relatively constant over this period (see table 1 and fig. 1 and 2). They were 34.8 g (31–40) for the male and 30.6 g (29–41g) for the female groups.

Mortality: During the period indicated a mean cumulative mortality of 11.3% was calculated for males and 22.4% for females, each with some variation between the studies. In males there was a slight decrease in mortality, whereas mortality rates in female mice increased remarkably ($p = 0.0009$) over time (see table 1 and fig. 3 and 4).

Incidences of selected tumor types

Benign and malignant tumors occurred in various organs with more or less wide variation from study to study. Neoplasms with incidences > 1% were found in the hematopoietic tissue, liver, lungs, Harderian glands,

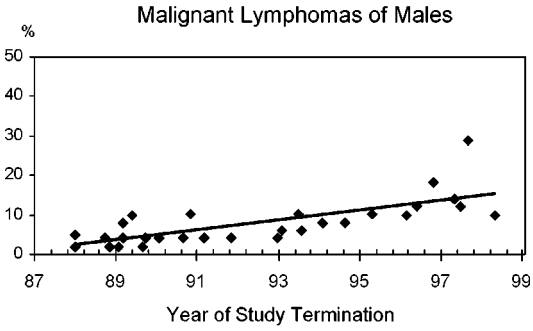


Fig. 9

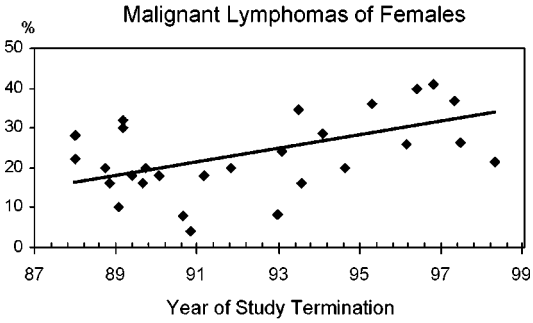


Fig. 10

Fig. 9–10. Results of the longitudinal analysis of malignant lymphoma incidences over 10 years. Data were received from $B6C3F_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each of the 29 studies and then plotted against the time point when the study was terminated.

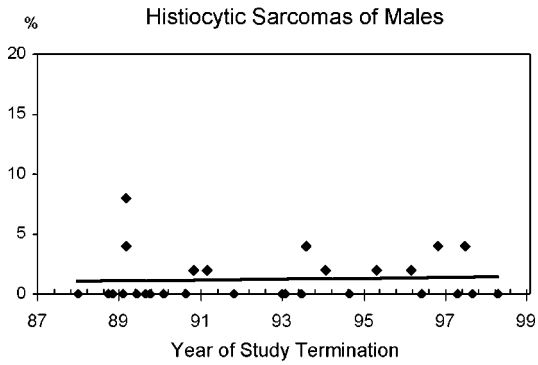


Fig. 11

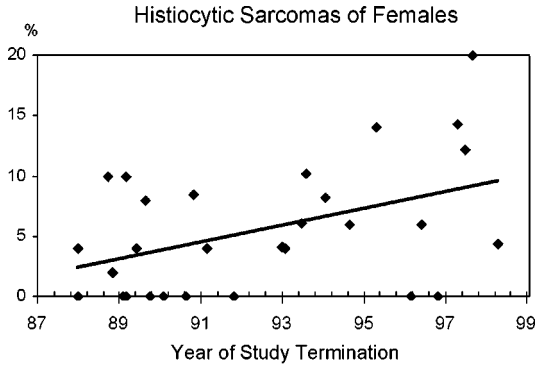


Fig. 12

Fig. 11–12. Results of the longitudinal analysis of histiocytic sarcoma incidences over 10 years. Data were received from $B6C3F_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each of the 29 studies and then plotted against the time point when the study was terminated.

vascular system, pituitary and thyroids (each females), adrenals (males) or reproductive organs of females. For reasons of comprehensibility the evaluation of tumors was limited to incidences below 0.1%.

Endocrinium

Pituitary: The most frequent endocrine tumor type was the adenoma mainly of the pars distalis in female mice with a mean incidence of 10.6%. In males the frequency of this tumor type was much lower and established at 0.8%. Over time examined no remarkable trend was observed for this tumor type.

Adrenal glands: Cortical adenomas (including subcapsular cell tumors) in adrenals occurred about ten times more frequently in males (2.4%) than in females (0.2%). They showed an increasing trend ($p = 0.0087$) over time in males (fig. 5). Benign medullary tumors, mostly of the pheochromocytoma type and cortical adenocarcinomas were seen in males and females with relatively low incidences (see table 2).

Thyroid/parathyroid glands and pancreas: In these organs neoplastic lesions were noted with low incidences which were slightly higher in females than in males (see table 2).

Reproductive organs and femal mammary gland

Female mammary glands: Adenocarcinomas of the female mammary gland occurred with a mean frequency of 0.5%.

Ovaries: Among the ovarian tumors the cystadenoma was the most frequent tumor (0.9%), which showed an ascending trend over time ($p = 0.0087$) as shown in figure 6. Other ovarian tumor types occurred with incidences of ≤ 0.6%.

Uterus/vagina: Among the uterine tumors, stromal polyps showed the highest mean frequency (1.9%), which increased over time ($p = 0.003$, see fig.7). There was also a slight ($p = 0.0396$) positive time trend in endometrial stromal sarcomas (see fig. 8), which occurred with a mean incidence of 0.4% (6% in one study and 0–2% in the remaining studies).

Testes: The mean rate of Leydig cell adenoma was 0.5% (see table 2).

Other organs

Hematopoietic system: In the hematopoietic system malignant lymphomas occurred with a mean frequency of 7.8% in males and 23.9% in females each with a high variation (4.5–52.0%). In males ($p = 0.0027$) and

Table 3. Frequency of tumors# in B6C3F$_1$ mice taken from 2-year studies: organs not belonging to the endocrinium or reproductive organs.

Sex **Organ** / Tumor Type[1)]		**Percentage Incidence**			
		Males		**Females**	
		Mean	Min.-Max.	**Mean**	Min.-Max.
Hematopoietic System					
– Histiocytic Sarcoma	m[&)]	1.2[*)]	0–8.0	5.5[*)]	0–20.0(+)
– Malignant Lymphoma, NOS	m	7.8[*)]	2–28.6(+)*	23.9[*)]	4.3–52.0(+)
– Leukaemia, NOS	m	<0.1		0.3	0–4.1
– Sarcoma, NOS	m	0		0.1	0–4.4
Liver					
– Hepatocellular adenoma	*b*[&)]	10.2[*)]	0–22.0	4.0[*)]	0–18.8(–)*
– Hepatocellular carcinoma	*m*	12.5[*)]	0–31.3(–)*	2.6[*)]	0–10.0(–)*
Lungs					
– Adenoma, NOS	*b*	8.9	0–20.4	2.7	0–8.2
– Adenocarcinoma, NOS	*m*	3.7	0–22.4	1.3	0–6.0
Harderian glands					
– Adenoma	*b*	5.6[*)]	0–12.2(–)*	5.1[*)]	0–14.0(–)*
– Adenocarcinoma	*m*	0.1	0–2.0	0.6	0–4.1
Vascular System/Mesenteric Lymph Nodes					
– Hemangio(sarco)ma	*b/m*	0.5	0–11.6	0.7	0–4.1
Vascular System/Other Locations					
– Hemangio(sarco)ma	*b/m*	3.1	0–5.3	3.5	0–10.1
Cecum					
– Leiomyoma	*b*	<0.1		0.2	0–2.1
Duodenum					
– Adenocarcinoma	*m*	0.4	0–4.1	0	
Jejunum					
– Adenocarcinoma	*m*	0.1	0–2.2	0	
Kidney					
– Renal Tubular Adenoma	*b*	0.1	0–2.0		
Stomach (Forestomach)					
– Squamous Cell Carcinoma	*m*	0		0.1	0–2.0
Bone					
– Osteosarcoma	*m*	0		0.3	0–2.1

[#)] occurring with a mean frequency > 0.1%; [*)] frequency of this tumor type evaluated by a longitudinal analysis (see Figures); [&)] *b = benign m = malignant;* [1)] tumors found in paired organs were counted as one tumor; *significant trend $p \leq 0.05$; (–) decreasing trend over 10 years; (+) increasing trend over 10 years

Table 4. Incidence and total number of tumors and tumor bearing B6C3F$_1$ mice.

		Males	**Females**
Mean Incidence of Tumors	%	60.8	77.6
Incidence Range of Tumors	%	32–102	42–132
Mean Incidence of Mice with Tumors	%	48.8	59.0
Incidence Range of Mice with Tumors	%	30–79	40–86
Number of Mice Examined		1446	1437

females ($p>0.05$) a positive trend was obvious over the time (see fig. 9 and 10).

Somewhat ascending incidences were also found for histiocytic sarcomas in females ($p>0.05$) which showed a mean incidence of 5.5% whereas in males (1.2%) they occurred without a trend (see fig. 11 and 12). Other systemic tumors occurred with low frequencies (see table 3).

Liver: Hepatocellular adenomas (males: 10.2%; females: 4.0%) and carcinomas (males: 12.5%; females: 2.4%) were found much more frequent in males compared to females (see table 3). In males carcinomas ($p = 0.0123$) and in females both, adenomas ($p = 0.0052$) and carcinomas ($p = 0.0019$) decreased over time as shown graphically in fig. 13 to 16.

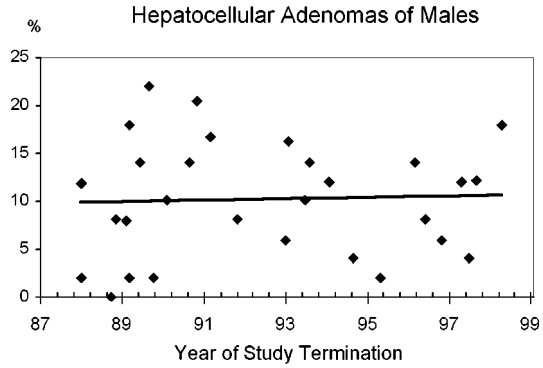


Fig. 13

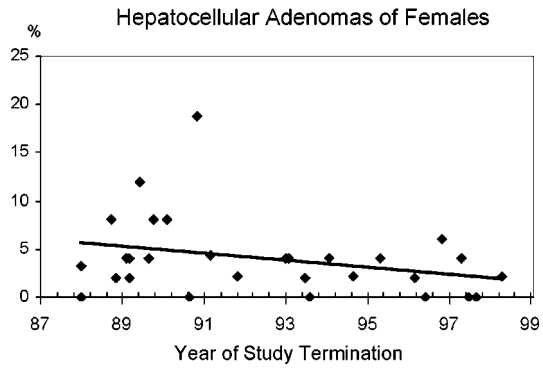
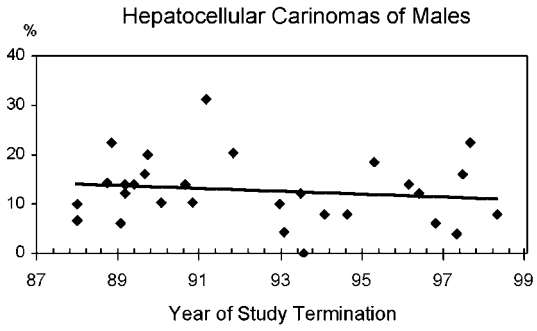


Fig. 14

Fig. 13–14. Results of the longitudinal analysis of hepatocellular adenomas over 10 years. Data were received from $B6C3F_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each collective and plotted against the time point when the study was terminated.



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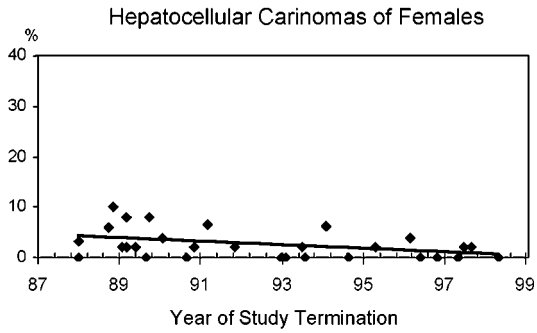

Fig. 16

Fig. 15–16. Results of the longitudinal analysis of hepatocellular carcinomas over 10 years. Data were received from $B6C3F_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each collective and plotted against the time point when the study was terminated.

Lungs: In the lungs bronchiolo-alveolar adenomas occurred with a mean frequency of 8.9% in males and 2.7.% in females, whereas lung carcinomas were present in 3.7% of males and 1.3% of the females. Both tumor types lack any time-dependent trend.

Harderian glands: Adenomas in the Harderian glands occurred in males and females with nearly equal frequency of 5.6 and 5.1% respectively and showed in both sexes a decreasing incidence trend ($p = 0.021$ and $p = 0.0377$) as can be seen from fig. 17 and 18.

Vascular system: Angiomatous tumors (hemangiomas, hemangiosarcomas) were found in mesenteric lymph nodes (males: 0.5%, females: 0.7%) and in various organs (males: 3.1%, females: 3.5%) mainly in the spleen.

Intestinal tract: In the stomach and intestines various mostly malignant tumors were noted each with a relatively low incidence.

Bone: Osteosarcomas in the bone were noted in females only (0.3%).

Kidneys: Renal tubular adenomas in the kidney occurred in males only (0.1%).

Other locations: In the remaining organs/tissues tumors occurred with incidences of less than 0.1% lacking any trend.

Incidences of overall tumors and tumor bearing animals

Overall tumors: The overall tumor incidences were 60.8% in males and 77.6% in females and varied over a relatively wide range (table 4). The longitudinal analysis revealed no remarkable time-dependent trend in the overall tumor incidence.

Tumor bearing animals: Among the 2885 mice 48.8% males and 59.0% females had developed a primary tumor. The study to study variation is very high (see table 4).

Discussion

The presented tumor data gives an overview over the survival rates and tumor spectrum of $B6C3F_1$ mice used at Bayer AG as control collectives in oncogenicity studies. The mean values and variation of these data gathered from 29 male and 29 female collectives are shown. Additionally, positive or negative trends in frequently noted tumor types and mortality data observed over 10 years are demonstrated. In some cases the data presented are suitable for a comparison with corresponding data published in the literature (see table 5).

Table 5. Mortality and incidences of selected tumors: comparison of own data with literature data.

Source of Data	A	B	C	D	E	F
Males						
Number of Animals Examined	1446	952	244	200	1791	1355
In Percent (%)						
Terminal Mortality	11.2	30.0	29	–	26	23
Number of mice Bearing a Tumor	48.8	–	70.9	50.5	–	–
Over all Tumor Rate	60.8	76.2	99	58.0	–	70.7
Pituitary: Adenoma	0.8	0.6	0.4	1.0	0.7	0.4
Adrenals: Cortical Adenoma	2.4	4.0	0.4	–	1.9	3.4
Thyroid Glands: Adenoma (follicular cells)	0.4	1.4	–	1.0	1.5	1.5
Harderian glands: Adenoma	5.6	–	–	1.0	2.7	4.7
Liver: Hepatocellular Adenoma	10.2	38.2	26.2	15.0	10.0	29.4
Hepatocellular Adenocarcinoma	12.5	20.4	23.8	9.5	21.1	17.9
Lungs: bronch.-alveolar Adenoma	8.9	18.1	9.0	10.0	12.1	16.0
bronch.-alveolar Carcinoma	3.7	7.6	4.9	0.5	4.9	5.1
Malignant Lymphoma	7.8	7.1	14.3	5.5	12.1	8.3
Histiocytic Sarcoma	1.2	0.4	–	3.5	–	0.5
Females						
Number of Animals Examined	1437	953	246	200	1791	1351
In Percent (%)						
Terminal Mortality	22.4	35.0	27	–	27	26
Number of mice Bearing a Tumor	59.0	–	57.3	41.5	–	–
Over all Tumor Rate	77.6	68.8	74.3	86.5	–	68.3
Pituitary: Adenoma	10.6	9.8	6.9	15.0	8.6	14.3
Adrenals: Cortical Adenoma	0.2	0.9	0.8	0.5	0.3	0.7
Thyroid Glands: Adenoma (follicular cells)	1.6	1.7	–	0.5	2.2	1.8
Harderian glands: Adenoma	5.1	–	15.5	1.4	3.3	
Uterus: Stromal Polyp	1.9	3.3	0.4	3.0	1.3	3.3
Ovary: Cystadenoma	0.9	2.9	–	–	–	1.9
Liver: Hepatocellular Adenoma	4.0	22.9	5.7	2.5	3.8	17.3
Hepatocellular Adenocarcinoma	2.6	10.9	4.5	4.5	4.6	8.4
Lungs: bronch.-alveolar Adenoma	2.7	5.6	3.3	9.5	4.9	5.9
bronch.-alveolar Carcinoma	1.3	3.8	2.0	2.0	2.0	2.4
Malignant Lymphoma	23.9	15.8	23.6	16.5	26.9	20.9
Histiocytic Sarcoma	5.5	1.7	–	5.5	–	1.4

A = mice used at Bayer AG (taken from Tables 1–4 of this paper); B = NTP Historical Control Information (2001) http://ehis.niehs.nih.gor/inp/docs/hpt_hcr.html; C = TAMANO et al. 1988; D = CHANDRA and FRITH 1992; E = HASEMAN et al. 1985; F = HASEMAN et al 1998; – = Data not available

Body weights and mortality

There was no remarkable increase in the terminal body weights of male and female B6C3F$_1$ mice. The mortality rates of 11.2% in males and 22.4% in females of these mice were lower than those of other stocks (see Table 5) although there was a distinct increase in the mortality of females during the last years.

Tumor data

Most tumors were found in the hematopoietic tissue, liver, lungs, Harderian glands, vascular system, endocrinium (pituitary, adrenals and thyroids) or female reproductive organs. The overall tumor spectrum was roughly in line with the tumor profile known for B6C3F$_1$ mice (CHANDRA and FRITH 1992; HASEMAN et al. 1985, 1998; TAMANO et al. 1988). Considering special tumor types there was a good correspondence between tumor incidences presented here and those reported by other authors (see table 5) except hepatocellular neoplasms and lung adenomas, which were found with lower incidences in male and female mice used at Bayer.

There was a remarkable decrease in liver carcinomas in both sexes and liver adenomas in females and also a significant negative trend in adenomas found in the Harderian glands in both sexes. Lymphomas (significantly in males only) and histiocytic sarcomas (females only) showed a positive tendency. Adrenal cortical adenomas increased significantly in males.

In female mice there were also significantly positive trends for uterine stromal polyps and stromal cell sarcomas as well as for ovarian cystadenomas. Concerning the re-

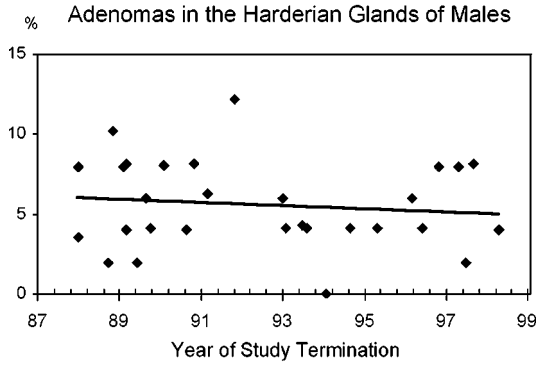

Fig. 17

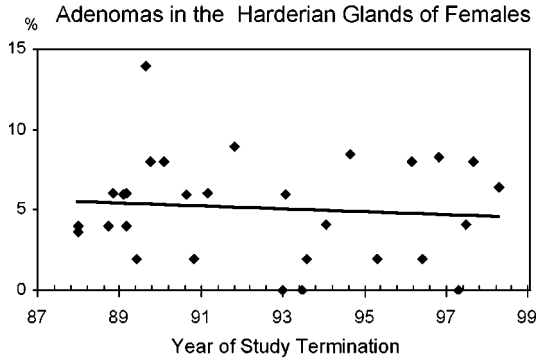

Fig. 18

Fig. 17–18. Results of the longitudinal analysis of adenomas in the Harderian glands over 10 years. Data were received from B6C3F$_1$ mice used as controls in 29 two-year studies terminated between 1988 and 1998. Group incidences (%) were calculated for each of the 29 studies and then plotted against the time point when the study was terminated.

maining tumor frequencies the overall tumor incidence and the number of tumor bearing animals B6C3F$_1$ mice used at Bayer showed no time trends and were comparable with corresponding data reported in the literature (see Table 5).

In conclusion, there was a significant increase in mortality of female mice most probably due to a distinct increase in malignant systemic tumors and some tumors of the reproductive organs. The frequency of hepatocellular tumors had decreased over time and the over all tumor incidence of females remained roughly constant.

In male mice a slight decrease in mortality might be caused by the fact that the incidence of hepatocellular carcinomas, the most frequent malignant tumors in males, had decreased significantly.

Since there were roughly constant terminal body weights of B6C3F$_1$ mice used at Bayer AG during this period and diet composition was unchanged the increasing or decreasing tumor incidences shown cannot be related to changes of the body weight or nutrition. Results shown in this paper and also data received from Wistar rats used at Bayer AG (EIBEN and BOMHARD 1999) demonstrate that there is no general rule for the assumption that changes in tumor incidences observed in rodents are mainly caused by body weight increase. Other labs had reported negative or positive relationships between increase in body weight, mortality and food intake and changes in frequencies of special tumor types for some rat strains (GRIES and YOUNG 1982; HASEMAN and RAO 1992; HASEMAN et al. 1997, 1998; RAO et al. 1990 a; ROE et al. 1995; ROSS et al. 1970; SEILKOP 1995; TURNBULL et al. 1985) and a causal relationship between increased body weights, and higher liver tumor incidences for B6C3F$_1$ mice (SEILKOP 1995 and HASEMAN et al. 1997). Therefore, feed restriction was discussed by several authors for rats KEENAN 1998; KEENAN et al. 1998; CHRISTIAN et al. 1998; HART et al. 1995; SINHA et al. 1988) and mice (SHELDON et al. 1995 and TURTURRO et al. 1996) to prolong survival of experimental animals. As shown by the data reported here, there is no necessity to practice feed restriction to B6C3F$_1$ mice used in our lab as a prevention for a too short life span. It is concluded that in the case of B6C3F$_1$ mice used at BAYER AG other factors such as a genetic drifts might be responsible for the positive or negative trends reported.

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