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History, Survival, and Growth Patterns of B6C3F1 Mice and F344 Rats in the National Cancer Institute Carcinogenesis Testing Program

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History, Survival, and Growth Patterns of B6C3F1 Mice and F344 Rats in the National Cancer Institute Carcinogenesis Testing Program. CAMERON, T. P., HICKMAN, R. L., KORNREICH, M. R., AND TARONE, R. E. (1985). *Fundam. Appl. Toxicol.* **5**, 526–538. The history of, and rationale for, the selection of the hybrid B6C3F1 mouse (C57BL/6 female × C3h/He male) and the inbred F344 rat for National Cancer Institute (NCI) bioassays is described. Survival percentages at the end of 2-year tests and weight-gain patterns during the tests of control animals are presented to guide investigators using these same animals in similar long-term experiments. Because information on a large number of animals (9385 mice and 10,023 rats) from a number of laboratories is presented, the conclusions should serve to give general guidance to investigators holding the same animals under a diversity of husbandry conditions. The program experience has been that the B6C3F1 mouse survival at the end of a 24-month study (25.5 months of age) is 80%; the F344 survival for the same period is 75%. This contrasts with the generally held assumption that rats are longer lived than mice. For the period of time from which animal data were collected, there was a progressively slight decrease overall in survival percentage. This observation cannot be explained, and contravenes the expectation that methodological improvements in producing the animals and marked physical improvements in the testing laboratories should have resulted in improving the survival. Weight gain patterns have a distinct and somewhat similar sexual dimorphism for both rat and mouse. The males of each species grow much faster initially and then essentially level off. The female mouse grows slowly and steadily, and by 2 years of age almost equals the male; the female rat shows the same steady gain, but is much lighter than the male at 2 years of age.

Good baseline data for growth and longevity of animal models are essential for design and interpretation of chronic rodent studies. There is little information published on the normal physiological parameters of many of the inbred strains, hybrids, and outbred stocks utilized in laboratory animal experiments. Data based on single experiments with small numbers of animals may not have general applicability because of gradual genetic drift and the wide variety of husbandry conditions encountered subsequently in other laboratories using these data. We present survival and weight gain data from the Carcinogenesis Testing Program, National Cancer Institute (NCI), on vehicle-treated and untreated control animals of two species—the hybrid B6C3F1 mouse (C57BL/6 female × C3H/He male) and the inbred Fischer 344 (F344) rat. These animals have been used in the standard carcinogenesis bioassay for several years. Their original selection was based on much less information than is now available.

HISTORY

The F344 rat strain was initiated by Maynie Rose Curtis in 1920 (Lindsey, 1979) in re-

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sponse to the needs of the Crocker Research Institute of Columbia University for a reproducible animal model for cancer. Development of the strain was continued principally by Dr. Wilhelmina Dunning, who needed inbred strains for chemotherapy research involving tumor transplantation. Dunning considered the F344 strain preferable to other inbred strains because of its small size and good breeding characteristics. F344 rats mature early and are very fertile, producing litters of 10 to 12. Dunning believed at the time that F344 rats had a relatively low incidence of spontaneous tumors (W. Dunning, 1983, personal communication). Dunning gave breeding stock to several commercial breeding laboratories and to Walter Heston of NCI. In 1951 Heston gave the F_{51} generation to the Veterinary Resources Branch, Division of Research Services, National Institutes of Health (NIH).

From the early 1960s, Fischer rats from the NIH colony were used at NCI for research (Yamamoto and Weisburger, 1977) and this led to the strain later being considered for carcinogenesis bioassays. Sprague–Dawley, Osborne–Mendel, and ACI rats were also considered by the NCI. The Sprague–Dawley rat was utilized for a period of time in a systematic evaluation of the mammary cancer endpoint as a relatively rapid limited *in vivo* bioassay for chemical carcinogens. However, the genetic variation of an outbred stock was considered to be a detriment, so the use of the Sprague–Dawley rat was finally discontinued. The Osborne–Mendel strain had been used extensively during the 1950s by Fitzhugh, Lehman, and Nelson at the U.S. Food and Drug Administration, especially in assaying halogenated hydrocarbons, and was eventually in limited use by NCI in testing members of that chemical class. The Fischer rat was thought to be a more sensitive model for chemical carcinogenesis than the ACI rat, principally because liver tumors developed within a relatively short latency period in Fischer rats exposed to chemical carcinogens, and the Fischer rat also appeared to give a consistent response to a number of different chemical classes (R. S. Yamamoto, 1983, oral communication).

From various studies of the NCI Bioassay Segment (previously the Carcinogen Screening Section), the Fischer rat appeared to be the preferable strain for testing of chemical carcinogens because of its sensitivity to various chemical classes, its reltively low spontaneous tumor rate (except for testicular Leydig cell tumors), and its small size, good breeding characteristics, and relatively easy maintenance. For these reasons, in 1970 the Fischer 344N rat, by then past its 80th generation, was adopted as the standard strain of rat for general bioassay purposes (J. Weisburger, 1982, personal communication).

The B6C3F1 hybrid mouse was developed for use in a carcinogenesis screening study of approximately 130 pesticides and industrial chemicals by NCI. The concept for this first large-scale carcinogenesis screening project was developed by Paul Kotin and Hans Falk in the early 1960s. Two F_1 hybrid mice, from three inbred strains—the B6C3F1 (C57BL/6 female × C3H/ANf male) and the B6AKF1 (C57BL/6 female × AKR male) (Bionetics, 1968; Innes *et al.*, 1969)—were used in this study. Hybrids were used rather than inbred strains for the increased hardiness and longevity characteristics of heterosis and because the wider genetic spectrum would more closely represent the genetic diversity of human populations. The C57BL/6 strain was selected because of its wide use, low incidence of leukemia (Miller, 1961), and long life span. Although results of longevity studies may vary due to environmental conditions, the C57BL/6 strain is consistently high ranked for longevity (Myers, 1978). The decision to use C3H males, rather than females, was made because C3H females have incidences of mammary tumors, as a result of mammary tumor virus (MTV) infection, as high as 97% at a mean age of 9 months (Bittner, 1958; Hummel, 1960; Squartini, 1961; Taylor and McKenna, 1961). Although higher in breeders, incidence is extremely high in both virgins

and breeders. For example, one report indicates a mammary tumor incidence for C3HeB/J females of 95% in breeders and 88% in nonbreeders (Hoag, 1963). F_1 hybrids of reciprocal crosses between strains of mice with high and low incidences of mammary tumors have a high incidence of tumors when born to and raised by high mammary tumor strain mothers, but are relatively free of mammary tumors when born to and raised by low-tumor strain mothers (Richardson and Hummel, 1959). The disadvantage of using C3H sires, however, is that C3H males have high incidences of liver tumors (Heston *et al.*, 1960). It is well known that incidences of MTV-induced tumors can be markedly reduced by foster nursing litters born by cesarean section; however, such C3Hf lines retain a high genetic susceptibility to mammary tumors (Heston and Deringer, 1952; Bittner, 1958; Hoag, 1963; Heston *et al.*, 1964; Staats, 1980). The AKR strain had been widely used in cancer research, but has a high incidence of leukemia by 8 months of age (Gross, 1958; Law, 1959; Hoag, 1963). The incidence of leukemia in the F_1 hybrids of crosses between high- and low-leukemia strains is usually intermediate. In several instances, however, extremely low incidences of leukemia have been observed in such crosses (Law, 1959). The hybrids developed for this study were expected to be hardy, long lived, and highly sensitive to chemical carcinogens. There were no previous data on the incidence of neoplastic or other diseases in these two hybrids (Bionetics, 1968; Innes *et al.*, 1969).

Results of this study indicated that both hybrids were appropriate for use in tests for chemical carcinogens. The decision to use the B6C3F1 hybrid, rather than the B6AKF1 hybrid, for the carcinogenicity testing program was not based solely on results of this study, however, as both hybrids had similar sensitivity to test chemicals and positive control substances. Despite the fact that the B6AKF1 mice had better survival of negative controls, fewer spontaneous tumors, and fewer nonneoplastic lesions in negative controls (Bionetics, 1968; Innes *et al.*, 1969), the decision to use B6C3F1 rather than B6AKF1 mice appears to have been based on knowledge that AKR mice are short lived, aggressive, and highly susceptible to leukemia (Gross, 1958; Roderick and Storer, 1960). Mean life spans of 22 inbred mouse strains raised in the same laboratory have been compared (Storer, 1966). Mean life spans ranged from 276 to 799 days for females and from 326 to 748 days for males. AKR mice of each sex had the shortest mean life span of the 22 strains, undoubtedly due to the fact that a 90% leukemia incidence in females and 81% leukemia incidence in males was observed. Because male AKR mice fought vigorously, it was necessary to cage them singly.

MATERIALS AND METHODS

The source of the B6C3F1 mice and Fischer rats included in this report changed several times. Initially, the production of a number of commercial breeders was purchased directly. By 1968, the Mammalian Genetics and Animal Production Section, Division of Cancer Treatment, NCI, had assumed total responsibility for production, utilizing its contracts with commercial suppliers who were supplied with breeding stock that had recently originated from the NIH in-house colonies.

In 1973, cesarean-derived lines, expanded and maintained behind a "barrier" in the NIH production facility, were supplied to stock a government-owned, contractor-operated facility. That facility was the main source of all animals from late 1973 until late 1976. From that time, all animals produced for the program came from behind two "strict barriers" maintained by commercial organizations. For additional protection, all breeders were introduced into the "barrier"-protected production rooms from associated-flora plastic isolators which were in turn stocked from an axenic population derived by each of the two commercial firms from NIH colony pedigreed starting stocks. At approximately 18- to 24-month intervals, restarts of pedigreed animals were delivered from the NIH foundation colonies for derivation and subsequent initiation of a replacement group of associated-flora isolators. The test animals were weaned at 3 to 4 weeks of age and shipped to the testing laboratory. Filtered shipping cartons were used after 1973.

The microbiological quality of the animals supplied to the test laboratories varied with time and breeder. In general, the quality improved as isolation procedures

were incorporated into the breeding program. The quality periodically would deteriorate as individual barriers were broken and undesirable microflora accidentally were introduced. At times it was not possible to identify these breaks before new bioassays had been initiated. Once started, studies usually were not stopped because of the presence of undesirable microflora.

Upon receipt at the test laboratories, the animals were quarantined for 14 to 18 days, therefore animals under study for 24 months represented animals that were approximately 25.5 months of age when the studies were terminated. Test and control groups were selected, and the groups were divided again into cage groups of five animals each. Both the group and cage selections were systematically randomized on the basis of body weight.

Both mice and rats were housed in plastic cages directly on commercially available hardwood litter materials; although initially they were not contractually required, some of the laboratories used nonwoven polyester filter covers in the early years. As new contracts were awarded over several years, all cages were eventually covered by filters. Most of the animals received water *ad libitum* via automatic waterers; some were supplied by water bottles. Anyone of three commercial rodent diets (Purina, Wayne, or Teklad) was used as feed. Room temperature and relative humidity ranges were 18.3 to 26.6°C and 30 to 70% except for a few excursions during the course of some studies. Means were approximately 21.6°C and 50% at most laboratories.

Survival data reported in this paper are derived from control groups of chemical carcinogenesis bioassays sponsored by the Division of Cancer Cause and Prevention, NCI. The data were received from eight contract laboratories for animals whose birthdates were up to and through 1977. Treated and untreated control groups were analyzed separately and in combination. Treated control groups received a variety of vehicle agents used to suspend or dissolve the subject compound. Corn oil, mixed in the feed or given by gavage, was the vehicle for almost 80% of the animals that received a vehicle required for the test compound. Table 1 lists the numbers of animals in the 4 principal vehicle/route groups and the percentages they comprise of the total vehicle group. The remaining 12.9% of vehicle recipients was scattered over 12 small groups (none of which exceeded 3% of the total and 7 of which were less than 1%). In addition to the vehicles of the 4 principal groups, acetone, buffered saline, steroid suspending vehicle, Tween 80, and trioctanoin were given alone or in combination. For 7 of the 12 groups, vehicle was administered by gavage or feed incorporation, and for 5 (comprising 3.2% of the total number of vehicle recipients) vehicle was administered by intraperitoneal or intramuscular injections.

The data presented in this paper are from all control groups which were under study for 24 months or longer. For these control groups, survival is reported as the percentage of animals alive after 24 months of study (i.e., at 25.5 months of age). Any control animals lost by planned interim sacrifice prior to 24 months on study were not considered to be at risk and were removed from the individual group total. Three laboratories in the earliest years of the testing program had survival rates so markedly lower than the rest of the program that their control groups for these years had to be deleted to give a fair representation of the true survival rates for the program as a whole. The 25.5-month survival rates of these deleted control groups are given by laboratory in Table 2.

Table 3 presents the final number and percentage of animals in each category (species, sex, and control type) for which survival data are presented.

Body weight data were obtained from the same control groups presented for survival analysis, with the addition of animals born through 1981. The observation times were weekly from 6 weeks of age (entrance on test) to Week 22, and thereafter at 2-week intervals up to Week 110. Actual numbers varied from one weighing to the next, depending on adherence to protocols and animal deaths. The average weights were derived from the weights of all animals weighed on a specific week of the

TABLE 1

SUMMARY OF THE NUMBER AND PERCENTAGES OF VEHICLE CONTROL ANIMALS FOR THE FOUR MOST COMMON VEHICLES/ROUTES OF ADMINISTRATION

Vehicle/route	B6C3F1		F344		% of total vehicle recipients
	Male	Female	Male	Female	
Corn oil/feed	518	918	1055	1103	56.8
Corn oil/gavage	310	395	357	325	21.9
Water/gavage	100	80	80	80	5.3
Water and carboxymethyl cellulose/gavage	50	50	50	50	3.1
Total					87.1

TABLE 2

SUMMARY OF ANIMALS DELETED FROM STUDY AND 25.5-MONTH SURVIVAL RATES OF DELETED ANIMALS

Laboratory		Years affected	Mice						Rats			
			Male				Female		Male			
			Untreated		Vehicle		Untreated		Vehicle	Untreated		Vehicle	
			No.[a]	%[b]	No.	%	No.	%	No.	%	No.	%	No.	%
A	Male	1971–1976	247	28.7	170	27.1	247	37.2	120	22.5				
	Female	1971–1974												
B		1971–1973	160	35.0	470	39.1					42	57.0	81	35.8
C		1971–1972												

[a] Number of animals deleted.
[b] Survival rate.

bioassay. For some laboratories, total weights of all animals in a cage divided by the number of occupants were accepted for groups being tested on compounds dosed either in feed or in water. The lack of individual animal weight data precludes the formal statistical comparison of weight curves from various control groups.

RESULTS

B6C3F1 Mice

The survival rates for female B6C3F1 mice are presented by year of birth and type of control in Table 4. There was little year-to-year variation in survival rates, with no evidence of heterogeneity in untreated controls ($p = 0.45$) and moderate heterogeneity in vehicle controls ($p = 0.03$). The overall survival rate for the vehicle control females (85.5%) was slightly higher than that for the untreated controls (82.3%). However, increased survival in vehicle controls was not observed in every laboratory or for every year of birth, and thus the overall increase was not significant in analyses stratified either by year of birth ($p = 0.21$) or by both laboratory and year of birth ($p = 0.51$).

The survival rates for male B6C3F1 mice are presented by year of birth and type of control in Table 5. Survival rates were much more variable in male mice than female mice, with marked year-to-year variation for both untreated controls ($p < 0.001$) and vehicle controls ($p < 0.001$). The overall survival rate for vehicle control males (77.8%) was lower than that of untreated controls (81.6%). The decreased survival in vehicle controls was significant in analyses stratified by year of birth ($p < 0.001$) and by both laboratory and year of birth ($p < 0.001$).

The overall survival rates were higher in female mice than male mice for both vehicle and untreated controls. The increase in survival in female mice was not significant for untreated controls in analyses stratified by year of birth ($p = 0.57$) or by both laboratory and year of birth ($p = 0.37$); however, the increase was highly significant in vehicle controls in analyses stratified by year of birth (p

TABLE 3

SUMMARY OF TOTAL NUMBER OF CONTROL ANIMALS, NUMBER DELETED BECAUSE OF ABNORMALLY LOW SURVIVAL, AND RESULTING PERCENTAGES OF ANIMALS USED TO COMPUTE SUMMARY SURVIVAL RATES

	Total No. in program	No. deleted	% of total utilized in analysis
B6C3F1			
Male			
Untreated	3634	417	89
Vehicle	1718	630	63
Female			
Untreated	3706	247	93
Vehicle	1741	120	93
F344			
Male			
Untreated	3225	42	99
Vehicle	1847	81	96
Female			
Untreated	3167	0	100
Vehicle	1907	0	100

< 0.001) and by both laboratory and year of birth ($p < 0.001$).

Body weight data were accumulated as groups of observations (individual or cage weight measurements) that were reported weekly through Week 22, and every 2 weeks thereafter. The distribution of the number of animals weighed as a function of time from the start of the experiments (animals at 6 weeks of age) is skewed, with the largest number of observations occurring at Week 24, followed by a long declining tail to the right, ending at 110 weeks of age. For Fig. 1 (males), Week 6 has 3153 observations (untreated and vehicle combined), increasing to 11,213 observations at Week 24 and gradually decreasing to 3155 at Week 110, the termination point of the experiments. Likewise in Fig. 2 (females), at Week 6 there are 3094 observations (untreated and vehicle combined), increasing to 11,417 observations at Week 24 and decreasing to 3366 observations at Week 110.

Male mice weighed approximately 20 g when put on test at 6 weeks of age (untreated—20.1 g; vehicle—20.8 g). They gained weight rapidly (approximately 1 g/week) until Week 20 (untreated—32.4 g; vehicle—33.6 g), and then gained gradually to a maximum at Week 80 (untreated—42.3 g; vehicle—41.5 g). Thereafter there was a very gradual loss in weight until Week 110 (untreated—40.5 g; vehicle—40.8 g). Because individual animal weights were not recorded, it cannot be determined if the declining weight curves after 80 weeks of age are due to weight loss in surviving animals or due to increased mortality in heavier animals. The weight curves for untreated and vehicle groups are essentially identical at all points.

TABLE 4

B6C3F1 FEMALE 25.5-MONTH SURVIVAL RATES BY YEAR OF BIRTH AND TYPE OF CONTROL

	Untreated		Vehicle		Combined	
Year	No.	%	No.	%	No.	%
1971	0	—	220	91.4	220	91.4
1972	645	84.3	571	86.3	1216	85.2
1973	934	82.3	130	79.2	1064	81.9
1974	455	83.3	250	86.8	705	84.5
1975	175	83.4	250	80.4	425	81.6
1976	500	81.8	50	88.0	550	82.3
1977	750	80.0	150	84.7	900	80.7
Total	3459	82.3	1621	85.5	5080	83.3

TABLE 5

B6C3F1 MALE 25.5-MONTH SURVIVAL RATES BY YEAR OF BIRTH AND TYPE OF CONTROL

	Untreated		Vehicle		Combined	
Year	No.	%	No.	%	No.	%
1971	0	—	58	91.3	58	91.3
1972	490	91.2	275	84.7	765	88.8
1973	900	79.7	120	75.0	1020	79.1
1974	455	81.3	250	74.8	705	79.0
1975	175	80.5	250	70.8	425	74.8
1976	457	74.8	0	—	457	74.8
1977	750	82.1	125	79.2	875	81.7
Total	3227	81.5	1078	77.8	4305	80.6

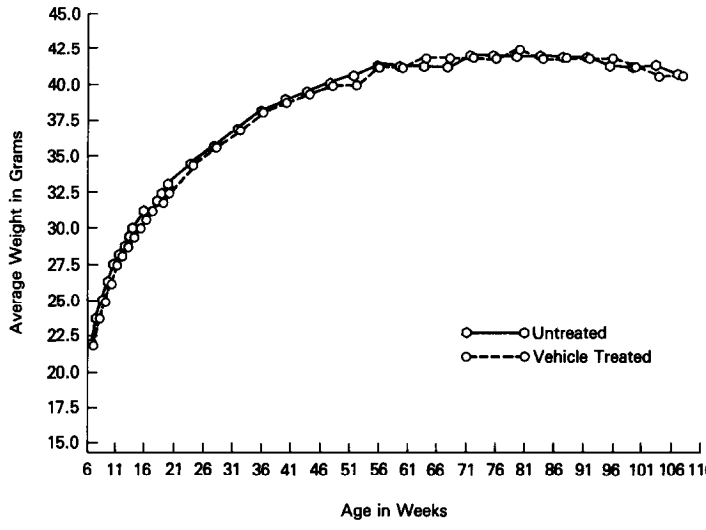

FIG. 1. Average weight of male B6C3F1 control mice for untreated and vehicle control groups.

The females grew less rapidly in the early weeks (Fig. 2), and they never reached a plateau as did the males. At 6 weeks of age they were approximately 2.8 g lighter than the males (untreated—17.4 g; vehicle—18.0 g). Unlike the males, beginning at Week 15 the vehicle groups gained less than the untreated groups, and this differential was maintained throughout the test period until at Week 110 there was a 4.2-g difference (untreated—41.8 g; vehicle—37.6 g). Because the females did not suffer a weight loss beginning at Week 80 as did the males, at Week 110 the untreated female groups weighed 0.7 g more than their male counterparts. However, the vehicle group females (obsd N = 1290) were 3.2 g lighter than the vehicle group males (obsd N = 1172) and the combined groups' average for each sex indicated that the males were 0.4 g heavier.

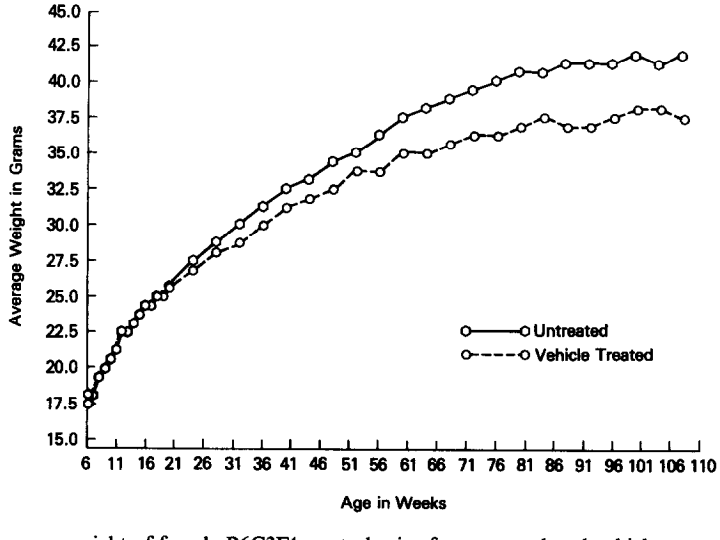

FIG. 2. Average weight of female B6C3F1 control mice for untreated and vehicle control groups.

F344 Rats

The survival rates for female F344 rats are presented by year of birth and type of control in Table 6. There was moderate year-to-year variation in survival in both untreated controls ($p = 0.07$) and vehicle controls ($p = 0.02$). The overall survival rate for vehicle control females (76.8%) was lower than that for untreated controls (80.4%). The decreased survival in vehicle controls was significant in analyses stratified by year ($p = 0.002$) and by both laboratory and year of birth ($p = 0.05$).

The survival rates for male F344 rats are presented by year of birth and type of control in Table 7. Survival rates were much more variable in male rats than female rats, with marked year-to-year variation for both untreated controls ($p < 0.001$) and vehicle controls ($p < 0.001$). The overall survival rate for vehicle control males (75.9%) was slightly higher than that for untreated controls (75.3%). This difference in survival was not significant in analyses stratified by year of birth ($p = 0.83$) or by both laboratory and year of birth ($p = 0.93$).

The overall survival rates were higher in female rats than male rats for both vehicle and untreated controls. The higher survival of female rats relative to males was highly significant for untreated controls in analyses stratified by year of birth ($p < 0.001$) and by both laboratory and year of birth ($p < 0.001$); however, the increase was not significant for vehicle controls in analyses stratified by year of birth ($p = 0.61$) or by both laboratory and year of birth ($p = 0.50$).

TABLE 6

F344 FEMALE 25.5-MONTH SURVIVAL RATES BY YEAR OF BIRTH AND TYPE OF CONTROL

	Untreated		Vehicle		Combined	
Year	No.	%	No.	%	No.	%
1971	105	81.9	81	79.0	186	80.6
1972	513	81.7	518	80.7	1031	81.1
1973	940	83.0	338	77.8	1278	81.6
1974	204	81.9	118	82.2	322	81.9
1975	225	80.4	550	74.2	775	76.0
1976	539	79.4	50	66.0	589	78.2
1977	691	76.1	202	71.3	893	75.0
Total	3217	80.4	1857	76.8	5074	79.1

TABLE 7

F344 MALE 25.5-MONTH SURVIVAL RATES BY YEAR OF BIRTH AND TYPE OF CONTROL

	Untreated		Vehicle		Combined	
Year	No.	%	No.	%	No.	%
1971	105	81.9	30	76.7	135	80.7
1972	495	80.0	477	84.9	972	82.4
1973	915	78.6	337	73.6	1252	77.2
1974	265	74.7	120	77.5	385	77.5
1975	225	67.1	550	72.2	775	70.7
1976	540	71.4	50	60.0	590	70.5
1977	638	72.3	202	71.8	840	72.1
Total	3183	75.3	1766	75.9	4949	75.5

Body weights were compiled as described for the mice. For males (Fig. 3), Week 6 has 2410 observations (untreated and vehicle combined), increasing to 10,630 observations at Week 24 and gradually decreasing to 4305 at Week 110. For the females (Fig. 4), Week 6 represents 2370 observations, Week 24 has 10,540, and there are 4545 observations for Week 110.

The male rats weighed approximately 103 g at Week 6 (untreated—101.1 g; vehicle—111.7 g) and tripled their weight by the 17th week (untreated—308.2 g; vehicle—310.3 g). They reached maximum weight at Week 76 (untreated—427.6 g; vehicle—435.7 g). By Week 110, their average weight had decreased by approximately 25 g (untreated—413.3 g; vehicle—394.5 g). This pattern of a loss of total body mass has been reported previously (Masoro, 1980). Weight curves for untreated and vehicle controls were essentially the same.

The male and female rats presented dimorphic growth patterns similar to those of the mice. The females were 14 g lighter than the males at Week 6 (untreated—88.4 g; vehicle—94.1 g). They proceeded to grow rap-

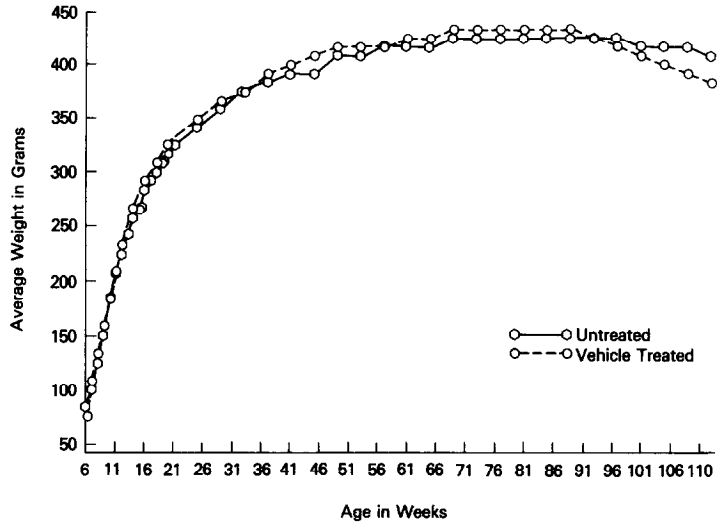

FIG. 3. Average weight of male F344 control rats for untreated and vehicle control groups.

idly until Week 19 (untreated—189.4 g; vehicle—191.5 g). Thereafter, their rate of gain slowed but never reached a plateau until they reached a maximum weight at 110 weeks (untreated—311.8 g; vehicle—280.2 g). A difference in weight gain between untreated and vehicle control groups began at Week 24 when they were equal [untreated—200.7 g (obsd N = 5792); vehicle—200.0 g (obsd N = 4748)] and continued until at Week 110 the untreated group was 31.6 g heavier (untreated N = 2816; vehicle N = 1729). The 31.6-g difference was 10% of the untreated group weight (311.8 g) and was consistent with the difference noted between the untreated and vehicle group weights of the female mice. The female mouse difference between untreated and vehicle-treated animals was 4.2 g, and that figure is also 10% of the weight of the untreated female mice (41.8 g).

DISCUSSION

The survival percentages presented are based on data from a program comprised of a number of testing laboratories with a spectrum of facilities and methodologies, and offer pragmatic baselines for judging the execution of chronic studies using this mouse hybrid and rat strain. Our data indicate that for the B6C3F1 mouse the survival rate for either sex should be close to or above 80% at the end of a 2-year study. Therefore, studies whose control groups are markedly below that figure are suspect as to execution. The F344 rat, in this program, demonstrated a slightly lower survival percentage. The survival rate of approximately 75% for either sex, though in a very acceptable range, is surprising in that the rat is commonly assumed the longer lived of the two species.

The criticism that 2-year rodent studies are "lifetime studies" which deal with extremely senescent animals with confounding spontaneous lesions (Salsburg, 1983) is countered by these high survival rates (B6C3F1—80%; F344—75%). It is misleading to classify a group of animals as being at the end of their life span when 75 to 80% are still alive. The decision made by the NCI staff in the early years of the bioassay program to standardize upon a 2-year study appears to have been justified by the data subsequently developed.

These data also have value in demonstrating that examining single studies with limited animal numbers could be misleading. Reports

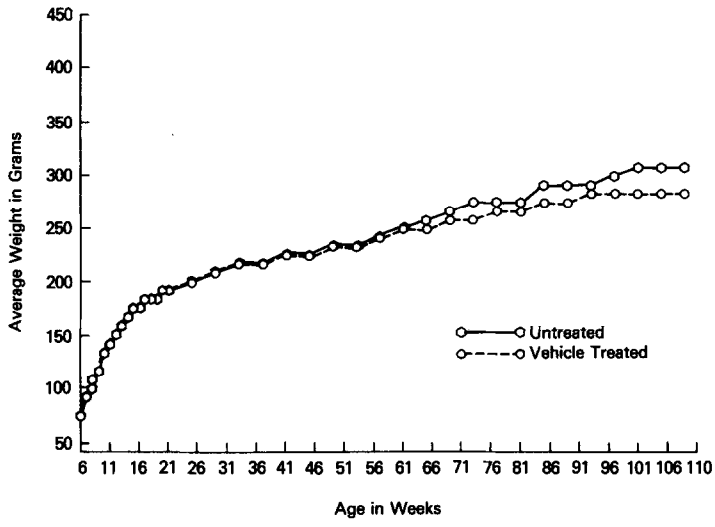

FIG. 4. Average weight of female F344 control rats for untreated and vehicle control groups.

of life span studies for the F344 rat strain, analyzed for survival rates at 25.5 months of age, show a wide spectrum of incidences. One study of male F344 rats (N = 572) bred and maintained within one research facility indicates a survival rate of approximately 30% at 25.5 months of age (Chesky and Rockstein, 1976). Another, designed to identify the impact of a restricted diet (Yu *et al.*, 1982) on males at 25.5 months of age, had approximately a 25% survival rate for the group (N = 260) fed *ad libitum*, and over 80% for the restricted group (N = 260). One overview (Hoffman, 1979) contained unpublished survival information from two separate colonies. In the first colony, at 25.5 months of age, female F344 rats (N = 139) had a 60% survival rate while the males (N = 153) had 70%. The second colony had three successive yearly male cohorts of 450, 1500, and finally 2400 animals born in and maintained in the same state of the art barrier facility. The initial cohort of 450 males had a 25.5-month survival rate of approximately 85%, the 1500 cohort had a rate of 78%, and the last 2400 animal cohort had a rate of 72%. The decline in survival from cohort to cohort is attributed to a buildup of undefined pathogenic flora within the single barrier in which the entire study was confined from beginning to end. Results of Sass *et al.* (1975) showing 75% survival (352 males and females combined) at 25 to 27 months of age appear to confirm our results.

The weight gain patterns present in our data for the B6C3F1 mouse reflect some aspects of sexual dimorphism. Although the patterns are different through much of the period on test, the final weights are almost identical. At Week 6 of life, the female's average weight (groups combined) is 86% of the male's average, and at Week 17 it is 77% of the male's average. By Week 80, the female's average is 92% of the male's, and by the end of the studies (110 weeks of age) the gap has closed so that the female's average weight is 98% of the male's.

The weight gain patterns of the F344 rat illustrate more distinctly the influence of sexual dimorphism. As in the case of the B6C3F1 mouse, the female F344 rat's average weight (groups combined) at Week 6 is 86% of the male's average weight, but by Week 17 has dropped to only 60% of the male's weight. Unlike the B6C3F1 female mouse, the female F344 rat never "closes the gap" and at Week 80 is only 63% of the male's weight, and 73% at 110 weeks of age.

This sexual dimorphism has implications for the design and conduct of experiments. Appraising for normal growth requires use of sex-specific growth charts. Cage size for experiments utilizing both sexes should be based on male growth patterns to ensure compliance with humane regulations.

We present these survival rates and weight gain patterns for the B6C3F1 mouse and the F344 rat to aid in evaluating bioassays performed under standard conditions with "conventional" animals maintained in a conventional manner. Although in all but the earliest years animals were produced within acceptable "barrier" facilities, outbreaks of viral and bacterial infection were not uncommon in the production facilities. Usually these outbreaks could not be detected before some affected animals were put on tests that could not conveniently be interrupted and restarted because of the long delays that would be required by contractual negotiations. The testing laboratories as a group made dramatic improvements in their facilities after 1974. The changes standardized the type and quality of husbandry which was provided but did not result in any major upgrading of classifications. They remained conventional facilities but with generally higher quality physical features and increased emphasis on animal care. Within the limits of the protocols, which necessarily had to give first priority to the containment of the test compounds and the protection of personnel, every attempt was made to protect the health of the animals, i.e., "barrier" facilities, frequent cage changing and washing, and so forth. These program changes, representing an upgrading of husbandry, were expected to improve the health profiles of individual animals and increase overall survival. However, despite year-to-year variation, there appears to be a slight decrease in survival over time for both sexes of both species. This is partially attributable to the censoring of very low survival groups from the early years. The introduction into the program of new laboratories having less experience with chronic carcinogenicity studies might also contribute to this decline.

Despite changes in management and scientific staff of the original Carcinogenesis Testing Program and its successors, B6C3F1 mice and F344 rats have remained the primary animal models used for chronic tests for carcinogenicity of chemical substances since initiation of the program in the early 1970s. Although program staff have repeatedly decided that these animals are appropriate for carcinogenicity testing, they cannot be considered necessarily ideal. Both B6C3F1 mice and F344 rats have been found to have a higher spontaneous tumor rate than was initially expected. Reviewing data on over 2500 B6C3F1 mice of each sex used as controls in NCI's Carcinogenesis Testing Program, Ward *et al.* (1979) found relatively high rates of liver tumors in males and of mammary gland tumors in females. Incidences of respiratory tract tumors in males and hematopoietic system tumors in females could also be considered high.

Although leukemia is not a particularly common lesion in most strains of laboratory rats, Fischer rats are highly susceptible to leukemia (Moloney *et al.*, 1970). Approximately 25% of F344 rats develop neoplasms of the lymphopoietic tissues, particularly large granular lymphocytic leukemia, also termed monocytic cell leukemia (Davey and Moloney, 1970; Sass *et al.*, 1975; Jacobs and Huseby, 1967; Sacksteder 1976). A review of 25 recent National Toxicology Program feeding studies (Haseman, 1983) found the incidence of leukemia and lymphoma in Fischer 344 control rats to be 25.5% in males and 19.6% in females. Other tumors occurring in Fischer 344 rats at relatively high incidences (over 19%) were mammary gland fibroadenomas and uterine endometrial stromal polyps in females, adrenal pheochromocytomas in males, and pituitary tumors in both sexes.

At the present time, the greatest advantage of using B6C3F1 mice and Fischer 344 rats for carcinogenicity testing is that they are well characterized and a great deal of histor-

ical data on spontaneous tumor incidence is available for them. Although it should be possible to develop strains with more "ideal" characteristics for carcinogenicity testing, toxicologists and regulatory decision makers may hesitate to rely on strains about which less background information is available.

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