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Fat, Frail and Dying Young: Survival, Body Weight and Pathology of the Charles River Sprague-Dawley-Derived Rat Prior to and Since the Introduction of the VAF^R Variant in 1988.

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Trends in survival and body weight were evaluated in 2140 control Sprague-Dawley-derived [Crl: COBS-CD(SD)BR and Crl: COBS-VAF CD(SD)BR] rats used for 24-month rat carcinogenicity studies between 1979 and 1991. Body weight and survival were remarkably stable in the CD-COBS rats used during 1979–1987: at 24 months, the mean survival in males was 68± 5%, and 60± 5% in females. With the CD-COBS-VAF rat, a variant of the CD-COBS strain used between 1988 and 1991, the survival at 24 months dropped to 41± 3% in males, and 44± 7% in females compared to the CD-COBS. The CD-COBS-VAF rat had a significantly reduced life span ($P<0.001$ at 24 months), a significant increase in mean body weight (males at 6 months : 672± 24 g *vs.* 536± 6 g; females : 359± 7 g *vs* 308± 3 g; $P<0.001$) and food consumption (males at 6 months : 31.3± 3.3 *vs.* 25.4± 2.1 g d^{-1} ; females : 22.0± 2.7 g *v.* 20.3± 2.0 g d^{-1} ; $P<0.001$). CD-COBS-VAF rats which failed to survive up to study termination had individual body weights at 3, 6 and 12 months which were significantly higher ($P<0.001$) than those which survived until 24 months. Our historical data base of control rats (CD-COBS and CD-COBS-VAF) in carcinogenicity studies revealed a significant (males:$P<0.001$; females:$P<0.01$) and inverse linear relation between mean 3-month body weight and 24-month survival. When compared to CD-COBS animals, CD-COBS-VAF rats showed an increase in the incidence of pituitary tumours in males, mammary fibroadenomas in females, an increase in the incidence and severity of glomerulonephrosis, and a greater incidence of animals which died without any obvious pathology. It is concluded that, in our Sprague-Dawley substrains, both the individual and the group mean body weights in early adult life appear predictive for the individual and group life expectancy. The decrease in longevity in the CD-COBS-VAF rat is principally due to disease and degeneration processes associated with fast growth and high body weight.

Introduction

Current guidelines for the carcinogenicity testing of chemicals, food additives and drugs recommend lifetime administration of the test compound to small rodents such as mice, rats or hamsters. The term 'lifetime' refers to a duration covering a major portion of the life span of the test animal population, i.e. a period of 18 to 30 months. Accordingly, European guidelines on the carcinogenicity testing of drugs in rodents lists a minimal study duration of 24 months for the rat and 18 months for the mouse and hamster.[1] Where the survival rate is high, the study may be extended up to 30 months in the rat and 24 months in the mouse, or for the life-span of the animals.[1] Current recommendations in the US specify a minimum test period of 18 to 24 months for mice and 24 to 30 months for rats. A study may be terminated prematurely when survival in control or low dose groups is 25–30%.[2] Similar limits for carcinogenicity studies have been specified in the OECD Guidelines on Testing of Chemicals.[3] In addition, most regulatory requirements for carcinogenicity studies in rats stipulate an overall, minimal survival of 50% after 24 months of study, or that a minimum number of 25 animals/sex/dose survives up to 24 months.[3–5] In

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summary, present regulatory guidelines are based on the premise that rats live longer than mice and hamsters, that a study duration of 24 months or longer represents an appropriate study duration in terms of life-span of current laboratory rats and, finally, that a 50% survival rate after 24 months of study is achievable with current laboratory rat strains.

However, while the life-span of the standard laboratory mouse strains has remained stable or has improved,[4,6,7] recent reports have described a decline in the longevity of several rat strains used in carcinogenicity testing, i.e. the Fischer 344,[8,9] the Sprague-Dawley[4,10] and the Long-Evans rat.[11]

In view of the regulatory requirements for adequate survival rates, this decline in the longevity of the principal rat strains used in carcinogenicity testing has initiated a general debate about the design of carcinogenicity studies of drugs, chemicals and food ingredients.[4] In the present report we have evaluated trends in survival, body weight and biological parameters of 2140 Sprague-Dawley-derived control rats, from carcinogenicity tests performed between 1979 and 1991. We present evidence for modifications in a number of biological parameters, particularly an increase in mean and individual body weight associated with a diminished survival of a Sprague-Dawley rat strain widely used in carcinogenicity testing and discuss the impact of these findings in terms of the present regulatory requirements for rat carcinogenicity studies.

Materials and methods

Animals

Sprague-Dawley-derived rats have been used in our laboratory for carcinogenicity bioassays for more than 10 years. The source of rats has been Charles River, France, supplying the Sprague-Dawley-derived Crl:COBS-CD(SD)BR rat (= 'CD-COBS') which we used up to 1987. The Charles River CD rat (official strain designation: Crl:CD(SD)BR) represents an outbred laboratory rat strain which was derived from the original Sprague-Dawley strain in 1955. The term 'COBS^R (=Caesarian Obtained Barrier Sustained) refers to animals that were originated by Caesarian rederivation and maintained behind a barrier against specific rodent pathogens. From 1988, we have used the Charles River Crl:COBS-VAF-CD(SD)Br rat (='CD-COBS-VAF'). The term 'VAF'^{R} (=Virus Antibody Free) is a variation of 'SPF' (Specific Pathogen Free) and refers to animals that show no evidence (by serology) of the presence of a specific list of rodent viruses.[12] CD-COBS and CD-COBS-VAF rats represent the same Sprague-Dawley rat substrain, except for the improved, certified sanitary status of the CD-COBS-VAF animal. The 'VAF'-sanitary status was introduced between 1985 and 1988 on a worldwide basis for most laboratory rodent breeding colonies of Charles River Laboratories. In France, the 'VAF' status for the Charles River breeding colony was introduced in 1988. In all carcinogenicity studies listed in this report, the age of the animals at study start was 4–5 weeks.

Diet

Between 1979 and 1991 rats in our laboratory were fed a commercial laboratory rodent diet, i.e. Diet A04C (Usine d'Alimentation Rationelle, Villemoisson-sur-Orge, France), a natural ingredient, cereal- and animal/vegetable protein-based rodent feed containing approximately 17% protein, 58% carbohydrates and 3% fat. In a single study (No 4, start date: 1981) the animals were fed B.P. Rat and Mouse No 1 (modified) expanded diet, a commercial natural ingredient laboratory rodent diet, manufactured by British Petroleum Nutrition Ltd., UK, containing approximately 15.4% protein, 60% carbohydrates and 3.5% fat.

Environmental conditions and test protocols

At the start of each carcinogenicity study, the animals were distributed randomly into three treated and respective control groups containing 50 to 55 animals/sex/group. Up to 1988, each study included a single male and female control group of 100 animals each. From 1988 to the present, we used two separate control groups of 50 to 55 animals per sex. The duration of all studies was 24 months. Rats were housed individually in transparent macrolon ('shoebox') cages on autoclaved softwood chip bedding. Treatment of animals was performed by oesophageal intubation (Studies No 10 and 11), or by the dietary route, that is, incorporated into the diet (all remaining studies). The respective control groups received vehicle or unmedicated diet. In the dietary studies, the rats were fed a powdered diet, while the studies conducted by daily intubation used a granulated diet. Animals were maintained at a mean room temperature of 20±3°C and a relative humidity of 60±20%. The average air change was 14 times per hour and the photoperiod was maintained at a 12-h light/dark cycle. The study protocols conformed to current requirements and recommendations for carcinogenicity testing of drugs issued by the EEC[1], the US Food and Drug Administration[13] and the Japanese Ministry of Health and Welfare.[5]

Animal use and care were in compliance with the current French and EEC regulations.[14] All studies were conducted in compliance with the Good Laboratory Practice Regulations as set forth by US Federal Regulations and by the Ministère des Affaires Sociales et de la Solidarité Nationale, France.

Recording of in-life data
DEC VAX computers were used to sort, treat and store all numerical data collected during the course of the studies.

Clinical observation, body weight and food consumption
Throughout the course of studies the animals were observed daily for survival, appearance and behaviour. They were weighed and their food consumption was measured at 1-week intervals.

Pathology
All animals found dead, sacrificed as moribund and terminal (24 month) survivors were subjected to a macroscopical examination. Selected organs were weighed and haemalum/eosin-stained, 4 µm sections from 38 different tissues were subjected to histopathological evaluation. In accordance with recommendations for comparison of pathological results of carcinogenicity studies,[15,16] the results of only two studies (Studies 9 and 10) are compared in this report; the two studies were conducted within a 3–4-year time period, and were evaluated by the same pathologist using the same terminology and diagnostic criteria.

Statistical evaluation
All the statistical tests used were two-tailed tests performed at the 5% level of significance. The Hartley test was used to assess homogeneity of variances. Owing to the different survival rate which occurred in studies 9 and 10, a statistical evaluation of the difference in incidence of tumours and non-neoplastic lesions was not performed.

Mean body weight comparison. Each sex was analysed separately. The mean body weight values for CD-COBS and CD-COBS-VAF populations are expressed as mean ± s.e.m. (standard error of the mean). The mean body weights of CD-COBS-VAF control groups were compared using one-way analysis of variance with the mean control body weight values of each individual carcinogenicity study as statistical unit. For body weight comparison of scheduled and unscheduled deaths in control groups, the statistical units were individual body weights of control animals.

Comparison of mean food consumption and clinical chemistry parameters. The mean food consumption of CD-COB and CD-COBS-VAF rats were compared for each sex using a Student's *t*-test on independent samples or an approximate *t*-test when variances were not homogeneous.

Evaluation of early adult body weight versus terminal survival (1979–1991). Regression analysis was performed using the SAS package (SAS System for Linear Models, 1986 Edition, SAS Institute INC., Cary, North Carolina, USA). Two weighted least-square regressions were performed for each sex separately, taking the 3-month mean body weights as independent variable. The dependent variable was the 18-month survival rate in the first regression and the 24-month survival rate in the second regression. In the regression analysis, the weight was the number of animals present in each group at the start of the study. Tests for the gradients equal to zero were performed and the *P*-values were calculated.

Results

Body weights
The mean 3- as well as the 6-, 12-, 18- and 24-month body weights of male control rats in carcinogenicity studies observed during the period 1979–1991 are shown in Table 1. In all studies, the mean male and female body weight increased during the initial 18 months of study, remained relatively stable thereafter or decreased slightly between 18 and 24 months. The mean body weight and the growth rate of the control CD-COBS animals used from 1979 to 1987 remained remarkably stable at each time point of the study. During this period, the mean body weight (± s.e.m.) of males was 462±4 g at 3 months and 679±8 g at 18 months. The male CD-COBS-VAF rats used between 1988 and 1991 were at each time point distinctly heavier than the CD-COBS animals: the mean body weight (± s.e.m.) of male CD-COBS-VAF animals at 3 months was 585±16 g at 3 months and 828±16 g at 18 months. The difference in early adult (age: 3 months) and late adult (age: 18 months) body weight between male CD-COBS-VAF and CD-COBS animals was highly significant ($P<0.001$).

Body weight data for the female groups are shown in Table 2. As in the males, the body weights of female CD-COBS-VAF rats were always significantly higher than those of female CD-COBS rats. The mean body weight (± s.e.m.) of female CD-COBS animals at 3 and 18 months was 265±3 and 446±8 g, compared to respective body weights of 317±5 and 480±12 g of female CD-COBS-VAF animals. This difference in early (age: 3 months) and late (age: 18 months) adult weight was highly significant ($P<0.001$). The body weight differences between CD-COBS and CD-COBS-VAF rats (both sexes) were somewhat more distinct at 3 months than at 18 months indicating that the CD-COBS-VAF rat gained weight faster as a juvenile or during early adult life. The increase in body weight of the CD-COBS-VAF rat was more marked in males than in females, resulting in a shift in the male:female body weight ratio from 1.6–1.7 in CD-COBS rats to 1.8–1.9 in CD-COBS-VAF animals.

Table 1 Body weight development [g] of male Crl:COBS-CD(SD) and Crl:COBS-VAF-CD(SD) control Sprague-Dawley rats in carcinogenicity studies performed between 1979 and 1991.

Study no.	*Year start*	*Number of animals*	*Body weight ± s.d. (g)* 3 months	6 months	12 months	18 months	24 months
			[Crl: COBS-VAF-CD(SD)Br Rat]				
1	1979	50	450 (a)	518 (a)	605 (a)	670 (a)	665 (a)
2	1980	100	480 (a)	535 (a)	604± 77	675 (a)	694± 90
3	1981	50	466± 39	544± 53	600± 70	682± 77	677± 80
4	1981	50	459± 39	547± 53	600± 67	697± 78	707± 86
5	1981	100	477± 41	543± 52	643± 67	698± 81	721± 99
6	1982	100	473± 44	553± 54	630± 70	720± 71	681± 92
7	1983	100	442± 49	555± 68	599± 81	662± 99	668± 104
8	1984	100	454± 40	511± 42	596± 65	657± 76	645± 95
9	1985	100	454± 40	515± 48	600± 64	647 82	664± 86
Mean± s.e.m.			462± 4	536± 6	613± 7	679± 8	680± 8
			[Crl: COBS-VAF-CD(SD)Br Rat]				
10	1988	55	624± 54	725± 71	829± 100	861± 114	808± 92
10	1988	55	630± 59	731± 74	831± 106	868± 140	770± 106
11	1989	55	575± 59	669± 78	763± 95	817± 105	783± 105
11	1989	55	599± 45	703± 60	805± 81	853± 119	828± 130
12	1990	50	540± 52	607± 57	723± 81	777± 101	733± 129
12	1990	50	539± 50	598± 63	717± 91	790± 100	715± 103
Mean± s.e.m.			585± 16	672± 24	778± 21	828± 16	773± 18

(a) Values not calculated

Table 2 Body weight development [g] of female Crl:COBS-CD(SD) and Crl:COBS-VAF-CD(SD) control Sprague-Dawley rats in carcinogenicity studies performed between 1979 and 1991.

Study no.	*Year start*	*Number of animals*	*Body weight ± s.d. (g)* 3 months	6 months	12 months	18 months	24 months
			[Crl: COBS-CD(SD)BR Rat]				
1	1979	50	245 (a)	295 (a)	330 (a)	445 (a)	450 (a)
2	1980	100	270 (a)	300 (a)	389± 56	470 (a)	465± 84
3	**1981**	**50**	260± 22	303± 36	366± 55	442± 70	452± 92
4	**1981**	**50**	275± 28	325± 41	395± 65	466± 86	506± 87
5	1981	100	273± 28	306± 36	380± 57	450± 77	483± 108
6	1982	100	268± 25	315± 40	384± 58	485± 55	475± 91
7	1983	100	261± 23	323± 38	370± 53	425± 73	428± 97
8	1984	100	268± 28	301± 33	363± 53	421± 71	426± 10
9	1985	100	264± 25	305± 33	355± 46	411± 64	432± 68
Mean± s.e.m.			265± 3	308± 3	370± 7	446± 8	457± 9
			[Crl: COBS-VAF-CD(SD)Br Rat]				
10	1988	55	324± 28	359± 34	424± 59	482± 75	481± 122
10	1988	55	324± 28	359± 34	424± 59	482± 75	481± 122
11	1989	55	319± 31	366 ± 47	449± 91	480± 89	497± 117
11	1989	55	327± 35	377± 48	443± 67	514± 89	503± 89
12	1990	50	300± 27	335± 35	386± 59	441± 87	448± 104
12	1990	50	301± 21	341± 31	387± 48	454± 69	477± 89
Mean± s.e.m.			317± 5	359± 7	423± 12	480± 12	483± 8

(a) Values not calculated

In both sexes, the variation of body weights at 3 and 18 months was greater in CD-COBS-VAF than in CD-COBS rats, as indicated by consistently larger standard deviations of the mean body weight values of CD-COBS-VAF groups. A comparison of the variances indicated a significant difference between CD-COBS and CD-COBS-VAF animals ($P<0.001$) and is consistent with a lesser degree of homogeneity in the CD-COBS-VAF variant strain. The difference in sample variation of CD-COBS-VAF, compared to CD-COBS, was more marked during the early adult stage than during the late adult stage which indicates that the growth pattern is more variable in CD-COBS-VAF than in CD-COBS rats.

Table 3 Comparison of the mean daily food consumption in control Crl:COBS-VAF-CD(SD) [study No 9] and Crl:COBS-VAF-CD(SD) [study 12] Sprague-Dawley rats at 3-, 6- and 12 months of carcinogenicity studies.

		Mean daily food consumption ± s.d. [g]				
Time	*Sex*	*CD-COBS*		*CD-COBS-VAF*		*Percentage increase in CD-COBS-VAF compared to CD-COBS*
		Number of Animals	*F.C.*	*Number of Animals*	*F.C.*	
3 Months	M	96	25.3± 2.1	91	33.3± 4.2***	32
3 Months	F	87	18.7± 1.7	86	21.6± 2.5***	15
6 Months	M	96	25.4± 2.1	98	31.3± 3.3***	23
6 Months	F	87	20.3± 2.0	73	22.0± 2.7***	10
12 Months	M	97	24.7± 2.7	94	29.7± 2.7***	20
12 Months	F	91	20.8± 2.3	87	21.7± 30*	4

*$P<0.05$
***$P<0.001$

Food consumption

A comparison of the mean daily food consumption in control CD-COBS-VAF rats at 3-, 6- and 12 months of studies 9 (start 1985) and 12 (start 1990) is shown in Table 3. Both investigations used powdered rodent diet. At all time points of both studies, the CD-COBS-VAF animals had a significantly higher daily food consumption than CD-COBS animals (males: +20 to +32%; females: +4 to +15%), the difference in food consumption between CD-COBS and CD-COBS-VAF rats was greatest during the first 6 months ($P<0.001$) and more marked in males ($P<0.001$ at 12 months) than in females ($P<0.05$ at 12 months).

Survival

The survival figures observed in our studies at 12, 18 and 24 months are shown in Table 4. In studies performed from 1979 to 1987 (i.e. the period we used the CD-COBS rat) survival at 24 months in control animals ranged from 58 to 75% in males (mean ± s.d.: 68±5%), and from 53 to 68% in females (mean ± s.d.: 60±5%). In five of nine studies, the 24-month survival of male CD-COBS rat was somewhat superior to that of the female (+9 to +20%); in the remaining studies the survival of males and females was comparable. Therefore, the overall mean survival in males and female CD-COBS was roughly the same order of magnitude.

However, in the studies initiated in 1988 and 1989 (10 and 11) using CD-COBS-VAF rats, the longevity dropped sharply: survival at 24 months in males ranged from 27 to 56% (mean ± s.d.: 41±13%), while for females the range was 35 to 51% (mean ± s.d.: 44±7%). The CD-COBS-VAF rat showed not only a reduced survival rate when compared to that of the CD-COBS rat, but, additionally, the survival rate of the genders changed. In four of six groups, male CD-COBS-VAF rats had a distinctly lower life span than females. The 18-month and 24-month survival rates of CD-COBS-VAF rats were significantly lower than the rates observed in CD-COBS animals ($P<0.001$ and $P<0.01$) for males and females at 18 months; $P<0.001$ for males and females at 24 months).

Table 4 Survival [%] of control Crl:COBS-CD(SD) and Crl:COBS-VAF-CD(SD) Sprague-Dawley rats in carcinogenicity studies 1979–1991.

Study No	*Year*	*Sex*	*Animals surviving (%) 12 months*	*18 months*	*24 months*
		[CRL:COBS-D(SD)BR rats]			
1	1979	M	96	92	70
	1979	F	98	86	56
2	1980	M	99	92	63
	1980	F	99	91	61
3	1981	M	98	96	58
	1981	F	100	96	58
4	1981	M	100	96	70
	1981	F	100	84	60
5	1981	M	97	90	67
	1981	F	99	91	68
6	1982	M	99	95	67
	1982	F	100	91	68
7	1983	M	97	93	69
	1983	F	100	87	60
8	1984	M	99	93	75
	1984	F	98	85	53
9	1985	M	98	93	74
	1985	F	97	93	54
Mean survival± s.d. [%] Males			98± 1	93± 2	68± 5
Mean survival± s.d. [%] Females			99± 1	89± 4	60± 5
		[CRL:COBS-VAF-CD(SD)-BR rats]			
10	1988	M	94	75	33
	1988	M	98	71	27
	1988	F	100	84	45
	1988	F	98	76	51
11	1989	M	94	80	47
	1989	M	91	62	27
	1989	F	96	69	36
	1989	F	96	67	35
12	1990	M	96	84	56
	1990	M	98	86	56
	1990	F	98	82	46
	1990	F	100	92	50
Mean survival± s.d. [%] Males			95± 2	76± 7	41± 13
Mean survival± s.d. [%] Females			98± 2	78± 9	44± 7

Poor survival in laboratory rats has been reported to be associated with elevated mean body weights.[8,17–19] To investigate whether decreased

Table 5 Comparison of body weight development [g] of two populations of male and female Crl:COBS-VAF-CD(BR) control rats in carcinogenicity studies. Control animals from studies 10, 11 and 12 were combined. ('Dropouts': animals found dead or sacrificed moribund prior to study termination. 'Survivors': animals surviving 24 months.)

Group	*Sex*		*3 months*	*6 months*	*12 months*	*18 months*
		n	188	184	180	133
Dropouts	M	mean	502.6***	666.8***	786.8***	829.9
		s.d.	50.9	80.0	104.6	136.0
		n	131	131	131	131
Survivors	M	mean	478.5	622.7	730.6	767.5
		s.d.	39.3	61.1	79.6	87.8
		n	182	181	176	134
Dropouts	F	mean	285.8**	349.6***	426.7***	480.9*
		s.d.	26.3	39.9	68.1	93.9
		n	138	138	138	138
Survivors	F	mean	278.2	335.1	394.8	459.4
		s.d.	25.4	35.0	55.8	75.6

*$P<0.05$
**$P<0.01$
***$P<0.001$

longevity within the same study was due to a shortened life span of those individuals which had higher body weights we compared the body weights of 3-, 6-, 12- and 18-month-old animals and of all the CD-COBS-VAF animals that died prior to study termination or were sacrificed as moribund ('Dropouts') in three carcinogenicity studies (studies 10, 11 and 12) to the corresponding mean body weights of those animals that survived up to study termination ('Survivors'). The values for male and female animals are shown in Table 5. The difference in mean body weight values of 'survivors' and 'dropouts' reveals that, on average, the 'survivors' were at all time points lighter than 'the dropouts'. The difference in adult body weights between 'survivors' and 'dropouts' was marked and highly significant, particularly in males ($P<0.001$ at the ages of 3, 6 and 12 months). The body weight differences between male 'survivors' and 'dropouts' decreased after 12 months and declined towards the later stages of the studies. This is likely to be a result of the shorter life span of the male 'dropouts' as, at the later stages of the study, the animal population tended to contain an increasing ratio of lighter animals.

Similar to the trend observed in the males, the females which survived up to 24 months were, on average, lighter than those that died prior to study termination. The main body weight differences between the female 'survivors' and 'dropouts' were significant at the ages of 3, 6, 12 and 18 months ($P<0.01$ and $P<0.001$, $P<0.0001$ and $P<0.05$, respectively). Therefore in both males and females, a high body weight during early adult life, that is from the age of 3 to 12 months, is predictive for the longevity of these animals.

In view of the significant relation between reduced longevity and elevated early adult body weight of individuals *within* a study, we determined whether a similar correlaton existed *between different studies*, including control groups of carcinogenicity studies with CD-COBS rats. The relationship between body weight and survival in control groups from all carcinogenicity studies was evaluated for the period 1979–1991. Using the data from Tables 1 and 2 (body weight development 1979–1991) and Table 4 (survival 1979–1991) mean 3-month body weights were plotted against the respective 18–month and 24-month survival rates. The results are shown in Figure 1. Statistical evaluation revealed a linear and highly significant trend for the correlation between male CD-COBS and CD-COBS-VAF mean 3-month body weights and their respective 18-month ($P<0.001$) and 24-month ($P<0.001$) survival rates.

As in males, in CD-COBS-VAF and CD-COBS females lower mean body weights are correlated with an increased life span: a statistically significant linear trend was found between an elevated mean body weight of control females at 3 months and reduced survival at 18 months ($P<0.01$), and after 24 months of study duration ($P<0.01$).

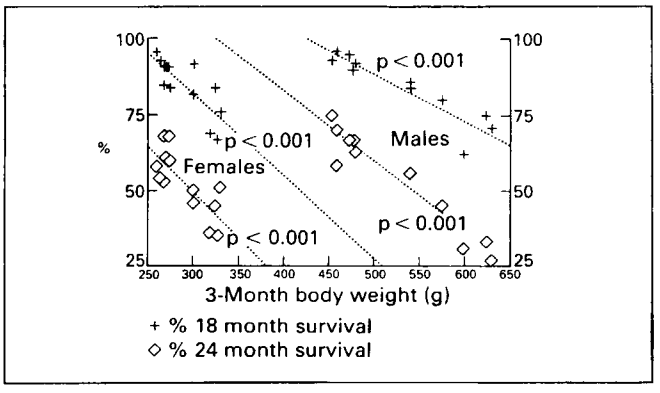

Figure 1 Mean 3-month body weights [g] of carcinogenicity control groups and their 18-month and 24-month survival rates [%]. Data from carcinogenicity studies performed between 1979 and 1991.

Pathology

Macroscopical findings. The spectrum of macroscopical findings was similar in studies 9 and 10. In study 10 (CD-COBS-VAF rat), a larger incidence of subcutaneous (i.e. mammary) growths and masses were recorded in female rats, and of pituitary masses in rats of both sexes. In addition, abundant body fat was frequently observed during necropsy of CD-COB-VAF animals, particularly in females.

Lesions in animals found dead or sacrificed as moribund. In study 10, using CD-COBS-VAF rats, we observed a far higher incidence (125/220) of animals which were found dead or were sacrificed as moribund than in study 9 using CD-COBS rats (72/200). While the ultimate cause for the death of these animals may not be ascertained, we attempted to determine the underlying pathological findings which in our view were the major factors leading to the death of these animals (Table 6):

(a) Severe renal glomerulonephrosis with associated lesions, such as secondary hyperparathyroidism ('metastatic' mineralizations, parathyroid hyperplasia, fibrous osteodystrophy) or uremic syndrome (gastric ulcerations, uremic pneumonia).
(b) Pituitary tumours, haemorrhagic and large enough to cause appreciable brain compression. This observation was generally associated with antecedent clinical signs such as lethargy and progressive weight loss. These tumours were frequently accompanied by gastric erosions or ulcerations and, in animals found dead, by pulmonary oedema.

In addition, in study 10 (CD-COBS-VAF rats) there were a number of animals, particularly males (14/78), which were found dead but for which no cause of death could be ascertained. These animals died without prior clinical signs, such as reduction in activity or food consumption and body weight loss and showed no major macroscopical or histopathological changes. This phenomenon occurred in animals of various ages.

Organ weights at terminal sacrifice. Comparing the organ weight results in studies 9 and 10, the mean relative male and female liver ($P<0.01$ and $P<0.001$, respectively) and kidney weights ($P<0.001$ and $P<0.01$) were significantly higher in CD-COBS-VAF rats than in CD-COBS rats (Table 7). The magnitude of relative liver weight increases was comparable in male and female CD-COBS-VAF animals (+20 and +24%, respectively), while kidney weight increases were more pronounced in male CD-COBS-VAF rats (+27%; $P<0.001$) than in females (+15%; $P<0.01$).

Tumour incidence. Tumour pathology data from studies 9 and 10 are shown in Table 8. The overall tumour incidence was compared on the basis of all animals (including unscheduled dead). Compared to the CD-COBS rat, both male and female CD-COBS-VAF rats showed a substantial increase (males: +37%; females: +43%) in tumour rates, despite the reduced longevity of this variant strain which may be expected to reduce the tumour

Table 6 Major pathological findings in control animals of study 10 found dead or sacrificed as moribund. Only categories of lesions with sufficient severity and incidence to have contributed to the death of these animals are listed.

	Males		*Females*	
Number of animals	*CD-COBS-VAF 110(a)*	*CD-COBS 100*	*CD-COBS-VAF 110(a)*	*CD-COB 100*
Total number of premature dead	78 (71%)	27 (27%)	59 (54%)	46 (46%)
Renal glomerulonephrosis	10 (9%)	1 (1%)	0	0
Pituitary tumours	27 (25%)	13 (3%)	35 (32%)	29 (29%)
Unknown causes	14 (13%)	1 (1%)	3 (5%)	0

(a) Two control groups of study 10 combined

Table 7 Comparison of selected relative organ weights from control Crl:COBS-CD(SD) [study 9] and Crl:COBS-VAF-CD(SD) [study 10] Sprague-Dawley rats of two carcinogenicity studies.

	Males		*Females*	
Number of animals	*CD-COBS-VAF 110(1)*	*CD-COBS 100*	*CD-COBS-VAF 110(a)*	*CD-COB 100*
Number at sacrifice	*32*	*73*	*51*	*54*
Liver	3.35± 0.88**	2.78± 0.74	3.69± 0.78**	2.99± 0.65
Kidney	0.49± 0.12***	0.38± 0.08	0.44± 0.11**	0.38± 0.09

(a) Two control groups of study 10 combined
***P<0.01*
****P<0.001*

Table 8 Tumour pathology [number of tumours/group] results from control Crl:COBS-CD(SD) [study 9] and Crl:COBS-VAF-CD(SD) [study 10] Sprague-Dawley rats of two carcinogenicity studies. Values in brackets are adjusted to a total number of 100 animals/group.

	Males		*Females*	
Number of animals	*CD-COBS-VAF 110(a)*	*CD-COBS 100*	*CD-COBS-VAF 110(a)*	*CD-COBS 100*
Hepatocellular tumours				
benign	6 (5)	**2**	1 (1)	**0**
malignant	4 (4)	**0**	0 (0)	**0**
combined (b)	10 (9)	**2**	1 (1)	**0**
Pituitary tumours				
benign	62 (57)	43	74 (67)	**60**
malignant	0 (0)	1	7 (6)	**8**
combined	62 (57)	44	81 (74)	**68**
Focal hyperplasia adrenal medulla Phaeochromocytomas	23 (21)	8	7 (6)	**5**
benign	10 (9)	**4**	1 (1)	**1**
malignant	2 (2)	**1**	0 (0)	**0**
combined	12 (11)	**5**	1 (1)	**1**
Mammary fibroadenomas				
Number of animals affected	0 (0)	**1**	63 (57)	**31**
Total tumour number	0 (0)	**1**	97 (88)	**37**
Total benign neoplasms	122 (111)	67	191 (174)	**100**
Total malignant neoplasms	29 (26)	33	50 (45)	**56**
Total number of tumours	151 (137)	100	241 (219)	**156**
Number of animals bearing tumours	92 (84)	72	106 (96)	**87**

(a) Two control groups of study 10 combined
(b) Benign and malignant neoplasms combined following the recommendations by McConnel et al. (1986)

incidence. The higher total tumour count in the CD-COBS-VAF rat was principally due to a marked increase (+90%) in the number of benign neoplasms with a concurrent decrease (approximately 20% in each sex) in the number of malignant tumours. The increase in benign neoplasia rates in the CD-COBS-VAF rat, (nearly doubled when compared to the CD-COBS), was mainly due to a high number of mammary fibroadenomas in females, and of pituitary adenomas in males. In addition, we noted in male CD-COBS-VAF rats a marginal increase in benign and malignant adrenal phaeochromocytomas, and in malignant hepatocellular neoplasms.

Non-neoplastic findings (all animals). The overall incidences of non-neoplastic histopathological findings in studies 9 and 10 were compared on the basis of all animals (including unscheduled dead). The principal difference between the two studies concerned the incidence of renal glomerulonephrosis (chronic progressive nephrosis) as shown in Table 9. When compared to CD-COBS animals of study 9, female CD-COBS-VAF rats had an increased incidence and severity of this lesion, while male CD-COBS-VAF rats showed an increased severity of renal glomerulonephrosis.

Discussion

The mature body weight, the longevity and the biological parameters of the CD-COBS Sprague-Dawley rat which was used in our laboratory in carcinogenicity studies from 1979 to 1987 have shown a remarkable stability. During this period,

Table 9 Incidence and severity of renal glomerulonephrosis in control Crl:COBS-CD(SD) [study 9] and Crl:COBS-VAF-CD(SD) [study 10] Sprague-Dawley rats of two carcinogenicity studies. Incidence in %. The severity of glomerulonephrosis was estimated using a three-scale grading system, i.e. grade 1: minimal to moderate, which includes focally affected kidneys; grade 2: marked; grade 3: severe.

	Males		*Females*	
Number of animals	*CD-COBS-VAF 110(a)*	*CD-COBS 100*	*CD-COBS-VAF 110(a)*	*CD-COBS 100*
No animals affected	105	85	75	41
Grade 1 (%)	38	**63**	49	37
Grade 2 (%)	30	**18**	15	4
Grade 3 (%)	25	**4**	4	**0**
Total (%)	95	**85**	68	**41**

(a) Two control groups of study 10 combined

we observed no discernible trend towards an increase in body weight or a decline in the life span of this strain. However, an abrupt change occurred with the CD-COBS-VAF rat: this variant strain showed a significant increase in food consumption and growth, resulting in a substantial increase of mature body weight. Parallel to the body weight increase we observed a drop in longevity, incidence of tumours and non-neoplastic lesions. All changes were more prominent in male CD-COBS-VAF animals than in females.

In our view, the close correlation of the decreased survival of the CD-COBS-VAF rat with the increase in body weight of this variant strain suggest a causal relationship between these two parameters. This is supported by the high statistical relation between increased body weight in early age and reduced longevity of individual animals (Table 5) and, again, by the linear relation of mean body weights and mean survival observed across our carcinogenicity studies (Figure 1). In agreement with a recent publication[19] we believe that, in males and in females of the Sprague-Dawley rat strain, a large body weight in early adult life and/or fast growth is an important marker for a reduced mean and individual survival. Moreover, the linear relationship between a high body weight in early life and the increased incidence of premature death suggests that even a minor increment in early adult body weight may lead to a significantly reduced life span, confirming that 'the weight of the animal in early life offers a crude measure of its length of life'.[20] The drop in longevity of the CD-COBS-VAF rat also correlates with the marked increase in food intake of this variant strain. At 3 months of study, the CD-COBS-VAF rats had an additional daily food consumption of 30% in males and 15% in females over respective values of CD-COBS animals. While this additional food consumption may partly be explained by the larger size of the CD-COBS-VAF animals, the male CD-COBS-VAF rat had also a distinctly higher food consumptions on a body weight basis, particularly during early adult life. The food consumption per body weight of female CD-COBS and CD-COBS-VAF rats is virtually identical. Given that 1 g of additional daily food intake in early adult life of rats may reduce the average life span by almost 4 weeks,[20] the low life span of the male CD-COBS-VAF is not surprising.

Our observation of the increased incidence of pituitary neoplasms, mammary fibroadenomas and glomerulonephrosis in the CD-COBS-VAF rat at terminal sacrifice and in animals which died during the study or were sacrificed as moribund is consistent with a report of Turnbull *et al.*[17] who reported for Sprague-Dawley rats an increased incidence in these parameters associated with high body weights. A similar increase of pituitary tumour, glomerulonephrosis and mammary fibroadenoma rates for the CD-COBS-VAF rat has been confirmed by other toxicology laboratories (R. A. Owen, Merck, Sharp and Dohm Laboratories, Riom, France personal communication, 1991) The drop in longevity of the CD-COBS-VAF rat is not unexpected as pituitary tumours and renal glomerulonephrosis are considered to be major life-limiting lesions in ageing Sprague-Dawley rats.[21,22]

The lower incidence of certain malignant neoplasms observed in CD-COBS-VAF animals is likely to be related to their reduced longevity, as the incidence of malignant neoplasms increases with age.

The elevated mean renal weight observed in CD-COBS-VAF animals is consistent with a report which described an association of severity of glomerulonephrosis and increased kidney weights.[23] From several factors that may have been the origin of the increased incidence and severity in renal glomerulonephrosis, increased food intake is probably the most important. While the question of which dietary component, i.e. protein, fat or caloric intake is responsible for the progression of this spontaneous nephropathy is still subject to controversy[24–25] the association between increased body weights and renal disease is well established.[17]

A substantial number (14/110) of CD-COBS-VAF animals, particularly males, were found dead during the course of our carcinogenicity studies without any obvious pathology. Death in the absence of discernible major lesions was rare in the CD-COBS rat, but appears to be common in CD-COBS-VAF animals: in our laboratory, we have occasionally observed death in the absence of pathologically detectable causes during 6-month toxicology studies using CD-COBS-VAF rats. As the terminal pathological evaluation in carcinogenicity studies is extensive, we do not believe that these deaths occurred from undetected disease processes. While involvement of cardiovascular events may be suspected, we have, however, not observed an increase in incidence or severity of cardiac lesions in the CD-COBS-VAF rat (data not presented in this report). Therefore, the origin of this phenomenon remains unclear.

Additional factors may have influenced the survival observed in the two recent carcinogenicity studies using the CD-COBS-VAF rat (studies 10 and 11): these two studies were performed via daily oesophageal intubation and used a granular diet, while all other studies used dietary administration and a powdered diet. However, our necropsy and histopathological observations indicate that in both studies the mortality due to gavage error was very low and accounted for less than 10 out of a total of 550 animals in each study.

This minimal impact of the administration route on mortality, growth or body weight is consistent

with observations in other toxicology laboratories (D. Brown, 1990; Hazleton Labortories U.K., personal communication; A. Wooley, Life Science Research, U.K., personal communication). In addition, our food consumption data from studies 10, 11 and 12 and several short-term studies in rats show that compound administration via daily gavage or via the dietary route and feeding a powdered or a granular diet produced no significant difference in food consumption or body weight gain. Therefore, we believe that the mode of administration does not account for the low survival or the marked increase in body weight which occurred in the CD-COBS-VAF animals of studies 10 and 11.

The change of body weight and survival in our CD-COBS-VAF rats resembles, superficially, the general drift towards a decreasing life span previously described for the CD and the Fischer 344 rat.[4,8,9,10,27] Our observations, however, are not consistent with a slow, gradual trend towards lower survival or increased body weight: in our laboratory, a period of remarkable stability of these parameters using the CD-COBS rat was followed by an abrupt shift. In our view, the marked increase in mean body weight and the drop in survival of the CD-COBS-VAF rat is more likely to be related to a change in innate factors of this variant strain. We can only speculate about the origin of these modifications. It has been suggested that the increase in mature animal weight in the current strains of laboratory rodents may be attributed to the elimination of infectious diseases allowing for a more complete expression of the genes controlling growth.[27,28] However, an improved health status would not explain why the abrupt body weight increase from the CD-COBS to the CD-COBS-VAF rat was marked in males and relatively minor in female CD-COBS-VAF animals. In addition, it appears reasonable to assume that the absence of certain infectious diseases would result in a prolonged life span rather than in a reduction of life expectation.[29] One could speculate that the CD-COBS-VAF rat represents a subpopulation of the original CD-COBS strain with predisposition for increased body weight and decreased survival.

Criteria of fecundity and fast growth are generally used as the basis for selection of breeding stock, thus discriminating against small animal size and small litters. Rats with good fecundity tend to be heavier.[27] CD-COBS-VAF rats appear indeed to be more fecund than CD-COBS animals: this has been confirmed by observations in our laboratory and other laboratories which reported a significant increase in litter size for the CD-COBS-VAF rat.[10] Laboratory rodents are bred mainly for short-term experiments and studies which necessitate a high 24-month survival rate use only a minor fraction of the total number of commercial laboratory rodents. Given that rat survival rates have remained sufficient until recent years, we feel that adequate longevity may have been taken for granted by the breeder and that the current CD-COBS-VAF variant may have been bred principally for fast growth and fecundity.

In addition to innate factors, nutrition possibly represents a further factor which may have contributed to the decreasing life span observed in the CD-COBS-VAF rat: following current regulatory guidelines for carcinogenicity studies,[3] the animals in our carcinogenicity studies are fed *ad libitum* a high-protein, high-fat commercial laboratory rodent feed, a diet designed to encourage rapid growth. It has been demonstrated for several years that an increased protein intake in early life, an enhanced efficiency in converting the food consumed to body mass, and a high level of food intake not only reduces longevity but also increases tumour incidence.[9,17,19,30,31,35,36] Thus the regulatory requirements for *ad-libitum* feeding of a high-fat, high-protein diet to a rat strain which may have a predisposition for a high food consumption, fast growth and obesity may have conspired to create large and overfed animals which finally die prematurely from tumours and degenerative diseases. Therefore, the low survival and high body weight of the CD-COBS-VAF rat may be a combined effect of a fast-growing rat strain with an outbred longevity potentiated by a nutrition which predisposes for fast growth and a shortened life span.

Within the frame of current regulatory requirements for carcinogenicity studies, the present life span of the CD-COBS-VAF Sprague-Dawley rat is no longer sufficient to meet the target of a minimal survival of 50% after 24 months of study. However, obtaining 25 survivors per group after 24 months of study[4] represents a more realistic target than a 50% survival rate – provided, however, that the initial animal number is expanded beyond the standard number used by the majority of toxicology laboratories, i.e. 50 animals/sex/dose. To meet the target of 25 survivors, it has been suggested that the initial group size in carcinogenicity studies is increased to 75 animals, resulting in a total number of up to 700 rats per study.[10] At present, this number may be even further inflated by requirements for additional animals used for pharmacokinetic monitoring.[32,33] However, whether an increase of the group sizes to ever escalating animal numbers provides for a satisfactory long-term solution of the current survival dilemma remains questionable.

A number of solutions for improving the survival of rats in carcinogenicity studies have been suggested, such as a reduction of the minimal study duration, the development of rat strains with improved longevity and limiting the

daily caloric intake by food restriction.[4,18] At a time, when regulatory requirements no longer reflect a biological reality, all aspects of current regulatory carcinogenicity bioassays must be reviewed including the minimal study duration of 24 months, the suitability of current laboratory rat strains, the composition of the rodent diets or the practice to of feeding *ad libitum*, none of which appears ideally suited for the performance of a meaningful *in-vivo* carcinogenicity bioassay.

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References

1 Council recommendation concerning tests relating to the placing on the market of medicinal products, Annex III, carcinogenic potential (1983). The Council of the European Communities 83/571/EEC. In: *The Rules Governing Medicinal Products in The European Community, Vol. III.* Luxembourg: Offfice for Official Publications of the European Communities, 1989.
2 Office of Science and Technology Policy: chemical carcinogens: a review of the science and its associated principles. *Federal Register,* 1984; **49**: and 1985; **50**:
3 OECD Guidelines for Testing of Chemicals No 451. *Carcinogenicity Studies.* Paris: Organization for Economic Co-Operation and Development, Environment Directorate, 1981.
4 Lang PL. Changes in life span of research animals leading to questions about validity of toxicologic studies. *Chemical Regulation Reporter* 1991; *Jan 18:* 1518–20.
5 Ministry of Health and Welfare, Japan. *1990 Guidelines for Toxicity Studies of Drugs Manual, New Drugs Division,* Japan: Pharmaceutical Affairs Bureau, 1990. pp. 60–74.
6 Allaben WT. The effects of caloric restrictions on spontaneous toxic end points and survivability in long-term rat studies. *The Toxicology Forum,* 1990. Transcript of the 1990 Annual Summer Meeting, Aspen, Colorado, USA 519–550.
7 Rao GN, Haeman JK, Grumbein S, Crawford DD & Eustis S. Growth, body weight, survival and tumour trends in (C57BL/6xC3H/HeN)F1(B6C3F1) mice during a nine-year period. *Toxicological Pathology* 1991; **18**: 71–7.
8 Rao GN, Haseman JK, Grumbein S, Crawford D, Eustis S. Growth, body weight, survival and tumour trends in F344/N rats during an eleven-year period, *Toxicological Pathology* 1990; **18**: 61–70.
9 Emmerson J. Industry experience on survival of the Fischer 344 rat. *The Toxicology Forum,* 1990. Transcript of the 1990 Annual Summer Meeting, Aspen, Colorado, USA, pp. 506–18.
10 Burek JD. Survival experience with the Sprague-Dawley-derived rat. *The Toxicology Forum,* 1990. Transcript of the 1990 Annual Summer Meeting, Aspen, Colorado, USA 453–481.
11 Szot RJ. Rat survival: influences and experiences. *The Toxicology Forum,* 1990. Transcript of the 1990 Annual Summer Meeting, Aspen, Colorado, USA, pp. 437–44.
12 Charles River France *'VAF – Le Standard International'* St.-Aubin-les-Elbeut, France: Charles River France, 1989.
13 US Department of Health and Human Services. Guidelines for the format and content of the non-clinical pharmacology/toxicology section of the application. Washington DC: Food and Drug Administration, Centre for Drugs and Biologics, 1987.
14 Directive du Conseil due 24 Novembre 1986 concernant le rapprochement des dispositions legislatives, reglementaires et administratives des etats membres relatives a la protection des animaux utilises a des fins experimentales ou a d'autres fins scientifique. *Journal Officiel des Communautes Europeennes* 1986; **L 358**: 1–28.
15 Haseman JK, Huff JE, Rao GN & Eustis SL. Sources of variability in rodent carcinogenicity studies. *Fundamental and Applied Toxicology* 1989; **12**: 793–804.
16 Haeman JK. Use of statistical decision rules for evaluating laboratory animal carcinogencity studies. *Fundamental and Applied Toxicology,* **14**: 637–48.
17 Turnbull GJ, Lee PN & Roe FJC. Relationship of body-weight gain to longevity and to risk of development of nephropathy and neoplasia in Sprague-Dawley rats. *Food and Chemical Toxicology* 1985; **23**: 355–61
18 Roe FJC. Letter to the editor. *Fundamental and Applied Toxicology* 1991; **16**: 616–18.
19 Roe FC, Lee G. Conybeare G *et al.* Risk of premature death and cancer predicted by body weight in early life, *Human and Experimental Toxicology* 1991; **10**: 285–8.
20 Ross MH. Dietary behaviour and longevity, *Nutrition Reviews* 1977; **35**: 257–65.
21 Squire AR, Goodman DG & Valeiro MG. Tumours. In: *Pathology of Laboratory Animals,* Benirschke K, Garner FM, Jones TC, ed, Vol II, chapter 12, p. 1228. New York: Springer Verlag, 1978.
22 Anver MR & Cohen BJ. Lesions associated with aging. In: *The Laboratory Rat, Vol I, Biology and Diseases,* chapter 14, p. 378. New York: Academic Press, 1979.
23 Owen RA & Heywood R. Age-related variations in renal structure and function in Sprague-Dawley rats. *Toxicological Pathology* 1986; **14**: 158–67.
24 Masoro EJ & Yu BP. Diet and nephropathy. *Laboratory Investigation* 1989; **60**: 165–7.
25 Bertani T, Zoja C, Abbate M, Rossini M, & Remuzzi G. Age-related nephropathy and proteinurea in rats with intact kidneys exposed to diets with different protein content. *Laboratory Investigation* 1989; **60**: 196–204.
26 Tapp DC, Wortham WG, Addison JF *et al.* Food restriction retards body growth and retards end-stage renal pathology in remnant kidneys of rats regardless of protein intake. *Laboratory Investigation* 1989; **60**: 184–95.
27 White WJ. *Issues in* Toxicology: Animal Quality, Consistency, Longevity, Fertility and Age/Weight Correlations, Transcript of the 3rd Short Course on

Laboratory Animals, Charles River, April 15 and 16. Paris, France, 1991.
28 Nolen GA. Effects of various restricted dietary regimes on the growth, health and longevity of albino rats. *Journal of Nutrition,* 1972; **102**: 1477–93.
29 Rao GN, Haseman JK & Edmondson. Influence of viral infections on body weight, survival, and tumour prevalence in Fischer 344/NCr rats on two-year studies. *Laboratory Animal Science* 1989; **39**: 389–93.
30 Ross MH, Lustbader ED & Bras G. Dietary practices in early life and spontaneous tumours of the rat. *Nutrition and Cancer* 1982; **3**: 150–67.
31 Roe FJC. Are nutritionists worried about the epidemic of tumours in laboratory animals? *Proceedings Nutrition Society* 1981; **40**: 57–65.
32 Weissinger J. Current and future regulatory policy on carcinogenicity testing – FDA policy. *Drug Information Association Meeting 'Carcinogenicity Testing: A Contentious Area in the Non-Clinical Safety Assessment of Medicinal Products'*, Montreux, Switzerland, September 16–19, 1991.
32 Bass R. Current and future regulatory policy on carcinogenicity testing – EC Policy. *Drug Information Association Symposium: Carcinogenicity Testing: A Contentious Area in the Non-Clinical Safety Assessment of Medicinal Products,* Montreux, Switzerland, September 16–19, 1991.
34 Haseman JK, Huff JE, Rao GN *et al.* Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57 BL/6N × CH3/HcN)F1 (B6C3F1) mice. *Journal of the National Cancer Institute* 1985; **75**: 975–84.
35 Tucker MJ. Factors influencing carcinogenicity testing in rodents. *Human Toxicology* 1987; **6**: 107–9.
36 Ross MH, Bras G. Influence of protein under- and overnutrition on spontaneous tumour prevalence in the rat. *Journal of Nutrition* 1973; **103**: 944–63.