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RELATIONSHIP OF BODY-WEIGHT GAIN TO LONGEVITY AND TO RISK OF DEVELOPMENT OF NEPHROPATHY AND NEOPLASIA IN SPRAGUE–DAWLEY RATS

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Abstract—The relationship of weight gain to survival, risk of development of chronic progressive nephropathy and risk of development of various neoplasms has been studied in the control groups from two routine chronic toxicity studies in Sprague–Dawley rats. The groups comprised 100 CFY strain rats of each sex observed up to the age of 109 wk and 120 CD strain rats of each sex observed up to 111 wk of age (females) or 121 wk (males). The eventual incidence of tumours was found to be related to body weight at several ages. There was also a statistically significant association between high body weight at various ages and increased mortality, particularly in the CD strain and particularly in females. The 'heavy' rats proved to have an increased risk of developing both progressive nephropathy and certain tumours. This relationship was particularly marked for pituitary tumours in both sexes and for benign and malignant mammary tumours in females, and was significant irrespective of whether tumours coexisting with marked or severe progressive nephropathy were classified as fatal or incidental. There was also some evidence that increased body weight was positively associated with risk of islet-cell tumours and lipomatous tumours in males and fibromatous tumours in females. The observations illustrate how non-specific factors, such as those that affect body weight, may profoundly influence mortality and tumour incidence in chronic toxicity studies. The findings also highlight the difficulty of classifying particular neoplasms as incidental or fatal where other potentially life-threatening pathology (e.g. progressive nephropathy) is present.

INTRODUCTION

Restricted access to food and changes in composition of the diet have long been known to influence survival, progressive nephropathy in the kidney and the incidence of certain tumours in laboratory rodents (Berg & Simms, 1960; McCay, Crowell & Maynard, 1935; McCay, Ellis, Barnes *et al.* 1939; Robertson, Marston & Walters, 1934). These effects of diet have been demonstrated repeatedly (Conybeare, 1980; Roe & Tucker, 1974; Ross & Bras, 1965 & 1971; Tucker, 1979; Yu, Masoro, Murata *et al.* 1982). It has been suggested that *ad lib.* access to an over-nutritious diet leads to endocrine disturbances, which favour tumour development (Roe, 1981).

Despite the higher incidence of tumours that results from unlimited feeding, this remains normal practice in the conduct of tests of chemical substances for toxicity and carcinogenicity. Variability within and between laboratories in control-animal tumour incidence in females exceeds the range expected on the basis of chance alone (Haseman, 1983). This constitutes a cause for serious concern since if there is similar variability in tumour incidence among treated groups, serious effects due to chemical treatment may be masked and non-specific differences in tumour risk may be wrongly attributed to treatment.

The work of Ross, Lustbader & Bras (1982 & 1983a,b) suggests a mechanism for variability in tumour incidence in control rats. Male rats were allowed to select their own diet, for quantity and formulation (choice of three formulae). The amount of food each rat consumed was notably consistent and preference for the type of diet changed only slowly. Rats that developed tumours could be distinguished from those that did not on the basis of the maximum body weight and the age at which that weight was achieved.

Body weights are recorded routinely in carcinogenicity studies comprising part of the safety evaluation of chemical substances. Thus, it is possible retrospectively to examine the relationship between body weight and the risk of tumour development. The present paper records the results of such a retrospective analysis of the data for control rats in two long-term studies.

METHODS

Source of data. Data for the analyses reported in this paper were taken from results for untreated Sprague–Dawley rats used as controls in two combined chronic toxicity and carcinogenicity studies begun in 1977 and 1978 at the same commercial

laboratory. In both studies the rats were similarly housed in mesh-floor cages and given free access to drinking-water and Spratts Laboratory Diet No. 2 (Spratts Patent Ltd, Barking, Essex). The environmental conditions were, nominally, an ambient temperature of 21 or 22°C, a relative humidity of 50% and a 12-hr light/dark cycle.

In the first study, the control group comprised 100 male and 100 female CFY strain rats (Anglia Laboratory Animals, Alconbury, Cambs). The rats were approximately 5 wk of age at the time their individual weights were first recorded, and they were observed until they died, up to a maximum of 109 wk of age. Most of them apparently contracted sialodacryo-adenitis at the age of about 7 wk, with clinically complete recovery during the following 2 wk.

The second study involved control groups of 120 male and 120 female CD strain rats (Charles River, Wilmington, USA) aged 5 wk at the time their individual weights were first recorded. The females were observed until they were 111 wk of age and the males up to 121 wk of age. At the age of 26 or 27 wk, the majority of rats showed signs of sialodacryo-adenitis. Most of them showed a temporary loss of weight while suffering from the disease, but they recovered completely with a rapid return to the normal weight-gain curve.

In both studies all rats were subjected to a standard comprehensive post-mortem examination and to extensive histopathological evaluation, but two different pathologists, who used somewhat different descriptive terms and criteria, were responsible for pathological evaluation in the two studies. Thus, in the study with CFY rats only 73 out of the 213 tumours seen (34%) were classed as fatal, whilst in the study with CD rats 266 of the 364 tumours (73%) were so classified.

Compilation of data. The data for individual animals were abstracted from the final reports on the studies for statistical analysis. Body weight data were obtained for CD rats at the ages of 5, 6, 7, 10, 18, 30, 54 and 78 wk and for CFY rats at 5, 6, 7, 9, 17, 29, 53 and 77 wk. For each animal and each of a range of tumour types selected on the basis of frequency of occurrence (Table 1), information was compiled for statistical analysis on lesions reported as having contributed to death (fatal) or not having contributed to death (incidental), and also on tissues not available because of autolysis. In addition, information on the presence and severity of progressive nephropathy and on week of death was abstracted for each animal. The severity of progressive nephropathy was graded on a four-point scale: none, early, moderate or advanced. The latter two grades were considered potentially fatal for the purpose of the analyses carried out.

Statistical analyses. The analyses examined the relationship between individual body weight at eight points in life and survival, risk of developing

Table 1. Numbers of untreated control rats with tumours in two separate studies in different Sprague–Dawley strains

		No. of rats with tumour*			
		CFY strain		CD strain	
	Sex ...	Male	Female	Male	Female
Tumour type	No. rats ...	100	100	120	120
Adrenal medulla					
—benign		11	1	5	1
—malignant		1	0	0	3
Total		12 (0)	1 (0)	5 (0)	4 (2)
Lipoma		7	4	21	4
Liposarcoma		1	0	0	0
Total		8 (0)	4 (0)	21 (16)	4 (0)
Skin/dermal fibroma		4	2	12	5
Subcutis fibroma		7	3	0	0
Mammary gland fibroma		3	3	0	0
Fibrosarcoma		2	8	16	11
Total		12† (3)	15† (4)	28 (16)	16 (12)
Pancreas					
—islet cell		7 (0)	3 (0)	24 (16)	3 (2)
Pituitary					
—adenoma		18	37	49	83
—carcinoma/adenocarcinoma		1	1	0	0
Total		19 (10)	38 (22)	49 (35)	83 (67)
Mammary gland					
—fibroadenoma		1	71	7	87
—adenoma		0	16	1	10
—adenocarcinoma		0	10	0	11
Total		1 (0)	81† (28)	8 (6)	92† (72)

*The pathological evaluation of the two studies (one in CFY strain rats and one in CD strain rats) were carried out separately by two different pathologists. The numbers of rats in brackets are those for which the tumour was considered fatal by the pathologist concerned with the study in the particular strain.

†Numbers do not add up because some rats had tumours of more than one type.

moderate or severe nephropathy and risk of developing the selected neoplasms. Separate analyses were carried out for each strain/sex combination, based on weight at each time point, and on three weight ratios: wk 30 weight/wk 5 weight, wk 54 weight/wk 5 weight, and wk 78 weight/wk 5 weight.

For each of these analyses, the individual weights (or weight ratios) were ranked in order and the four 'quintiles' (cut-off points that divide the ranked data into five equal numbers of observations) calculated. Rats with a rank below the lowest quintile were assigned to the 'light' weight group, those below the next lowest quintile to 'lightish', those below the next to 'medium', those below the next to 'heavyish' and those above the highest quintile to 'heavy'.

Animals that had died before the time point in question and therefore had no weight data were excluded. Additionally, animals that died within 8 wk following the time point in question were also excluded, to minimize the possibility of weight at that time point being affected by the presence of a tumour.

The statistical methodology used to take account of survival differences was based on the method described fully by Peto, Pike, Day *et al.* (1980). Briefly, for each strain/sex combination, the data for each of the five weight groups were subdivided into time intervals. Within each time interval, the number of tumour-bearing rats *observed* in each weight group was contrasted with the number *expected*, assuming no difference in tumour rate between the weight groups. The number of rats expected to develop tumours in a given time interval was calculated by multiplying the number at risk in a weight group by the tumour rate for all weight groups combined. The tumour rate (number with condition/number at risk) was calculated differently depending on whether the tumours were considered fatal or incidental. For fatal tumours, tumour rate was defined by the ratio (no. of animals dying in the interval because of the tumour of interest)/(no. of animals alive at the beginning of the interval), with 1-wk periods being used for the time intervals. For incidental tumours, tumour rate was defined by the ratio (no. of animals dying in the interval with the tumour of interest)/(no. of animals dying in the interval), with broader time intervals being used (to avoid small numbers at risk). The individual observed and expected numbers were summed over the time intervals to form a total observed (O) and expected (E) for each weight group. The deviations (D = O − E) represent departures from the null hypothesis that tumour incidence is unaffected by weight, and were combined over sexes and/or strains as appropriate.

For most analyses the original classification by the pathologist of tumours as fatal or incidental was used. However, for two representative points in the lifespan (wk 10 and 54) some additional analyses were carried out in which any tumours occurring in rats that died with marked or severe progressive nephropathy were classified as incidental.

Tests of significance were made by approximate chi-squared statistics both for overall between-weight-group variation in tumour incidence and also for trend, i.e. the tendency for age-specific tumour incidence to rise with increasing body weight. The trend statistic is the more informative and it is the probability based on it that is reported here.

The tables in this paper generally present results for the combined strains as these provide the most powerful test of the hypothesis of interest. Cases where significant differences were seen in only one strain are reported in the text.

RESULTS

Body weights

Body weights over the lifetime of the animals were within the range for untreated animals of the same strain in other studies performed at the same laboratory. The CD strain animals grew less rapidly and had a lower mature body weight than the CFY strain.

Mortality

Survival was generally higher in CD rats than in the CFY animals (Fig. 1). Mortality was positively related to body-weight gain. This was apparent in both CFY and CD strains, in males and females, and was evident at all eight age points (Table 2). The relationship was present from the age of 5 wk and was marked by 10 wk of age. It continued to be highly significant ($P < 0.001$) to the age of 54 wk in

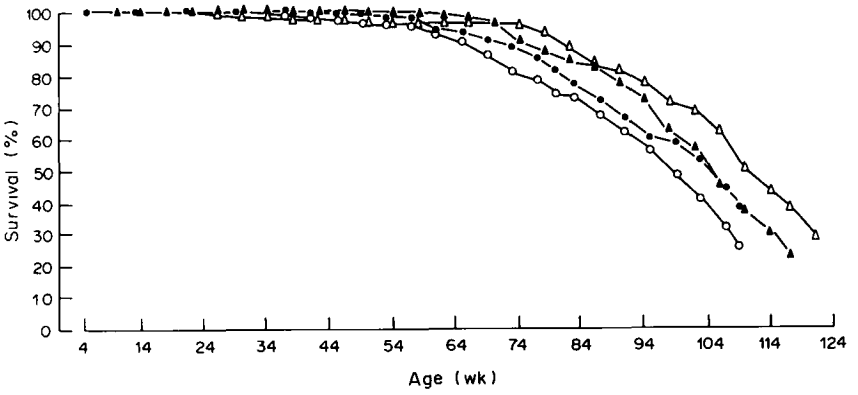

Fig. 1. Survival among untreated Sprague–Dawley CD strain males (△) and females (▲) and CFY strain males (○) and females (●).

Table 2. Significance of relationship between body weight or weight ratio and mortality in untreated Sprague–Dawley CFY and CD rats (data combined)

Age (wk)	Level of significance† Males	Females
	Body weight	
5	*	*
6	*	**
7	**	***
10	***	***
18	***	***
30	***	***
54	***	***
78	*	***
	Weight ratio‡	
30/5	*	**
54/5	*	***
78/5	(*)	**

†See Methods section for details of statistical analysis. Trend statistic: (*) $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
‡Ratio between weight at 30, 54 or 78 wk of age and weight at 5 wk of age.

males and 78 wk in females. The association with weight was more highly significant in females than in males and was particularly marked in female CD rats. This can be seen clearly in Table 3, which summarizes the results of an analysis comparing mortality among rats classified by body weight at wk 54. In the female CD rats none of the 47 animals in the heavy and heavyish groups survived until the end of the study, as compared with 27 survivors out of 72 animals in the other three weight groups combined.

Progressive nephropathy

Progressive nephropathy was found in 81% of male and 54% of female animals, with incidence similar in the CFY and CD strains. Both glomerular and tubular elements were affected. In both males and females there was a significant association between high body weight and progressive nephropathy. Analysis at the representative ages of 10 and 54 wk showed the relationship was initially more marked in males ($P < 0.001$) than in females ($P < 0.05$) but later the relationship was significant in both sexes ($P < 0.001$) and the weight-gain ratio at 54 wk of age was also significant in both males ($P < 0.01$) and females ($P < 0.001$).

Tumour incidence

The numbers of rats with the more common types of tumours in both sexes and strains are given in Table 1. In both studies, it was reported by the pathologist concerned that both a tumour and severe glomerulonephrosis were causes of death in particular animals. The numbers of rats with fatal tumours given (in brackes) in Table 1 are based on the pathologists' opinions, as are the results presented in Table 4 summarizing the significant relationships found between body weight and tumour incidence.

Pituitary tumours

Pituitary tumours were present in 68 male and 121 female rats, but only two of the tumours were regarded as malignant on the basis of histological criteria. The combined data for the two strains showed a strong relationship between body weight at

Table 3. Body weight at 54 wk of age and subsequent mortality among untreated Sprague Dawley CFY and CD rats

Statistic	Weight group...	Results Light	Lightish	Medium	Heavyish	Heavy
			CFY Males			
No. of rats*		18	18	20	18	19
Mean weight (g)		707	788	841	894	1005
Deaths†—(no.)		10	15	11	14	17
—(%)		56	83	55	78	90
Trend‡				$\chi^2 = 6.49$	$P < 0.05$	
			CFY Females			
No. of rats*		18	19	19	19	20
Mean weight (g)		390	450	501	559	665
Deaths†—(no.)		8	6	8	13	16
—(%)		44	32	42	68	80
Trend‡				$\chi^2 = 4.94$	$P < 0.05$	
			CD Males			
No. of rats*		23	23	23	24	23
Mean weight (g)		612	670	713	770	857
Deaths†—(no.)		7	9	13	12	15
—(%)		57	57	74	83	87
Trend‡				$\chi^2 = 8.46$	$P < 0.01$	
			CD Females			
No. of rats*		23	24	25	23	24
Mean weight (g)		332	364	400	434	508
Deaths†—(no.)		11	19	15	23	24
—(%)		48	79	60	100	100
Trend‡				$\chi^2 = 27.40$	$P < 0.001$	

*Surviving to wk 62.
†Before terminal kill.
‡Based on the method of Peto *et al.* (1980), taking into account time of death.

Table 4. Significance of relationships between body weight or weight ratio and the number of animals with tumours of various types, using combined data for untreated Sprague–Dawley CFY and CD rats

		Level of significance†						
	Tumour . . .	Pituitary		Mammary‡	Mammary adenocarcinoma	Fibromatous	Fibrosarcomas	Pancreatic islet cell
Age (wk)	Sex . . .	Males	Females	Females	Females	Females	Females	Males
				Body weight				
5			*	*				
6			**	***				
7			**	***				
10			**	***				
18		(*)	***	***	**			
30		**	**	***	*			*
54		**	***	***	***	*		**
78		**	**	***	**	**	*	
				Weight ratio§				
30/5		*	(*)	**		*		
54/5		**	**	***	(*)	**	*	*
78/5		*	(*)	***	(*)	**	*	(*)

†See Methods section for details of statistical analysis. Trend statistic: (*)$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
‡Excluding mammary gland fibroma.
§Ratio between weight at 30, 54 or 78 wk of age and weight at 5 wk.

various ages and pituitary tumour frequency in both males and females. This was significant from the age of 5 wk in females ($P < 0.05$) but not until 30 wk of age in males. Examination of the data of the two strains separately showed that the relationship was evident in CD strain females at the ages of 10 wk ($P < 0.01$) and 54 wk ($P < 0.001$) and in males at the later time only ($P < 0.01$). In CFY strain males and females the relationship was not significant at either age.

Mammary fibroadenoma, adenoma and adenocarcinoma

Mammary tumours of these types were significantly more common in heavy than in lighter females, with the relationship apparent from 5 wk of age ($P < 0.05$). The correlation was highly significant from the age of 6 wk to 78 wk of age ($P < 0.001$). In the CD strain females the association was significant at both 10 and 54 wk of age ($P < 0.001$) while in CFY females the significance was apparent at the later age only ($P < 0.05$). There were only nine males with mammary tumours and no correlation with body weight was found.

When analysis was restricted to mammary adenocarcinomas only, there was a significant correlation with body weight despite a total incidence of only 21 out of the 220 females. The correlation was evident from the age of 18 wk ($P < 0.01$) but none of the weight ratios was significant at the 95% confidence level. The relationship was significant in both CFY and CD strain females at 54 wk of age ($P < 0.05$ and $P < 0.01$, respectively).

Fibromatous tumours

In females the combined data showed a significant association between body weight and frequency of fibroma or fibrosarcoma. This was significant at the ages of 54 and 78 wk ($P < 0.05$ and $P < 0.01$, respectively) but all three weight ratios were significant. When the two strains were considered separately at two time points (10 and 54 wk), the only significant association found was at 54 wk of age in CD females ($P < 0.05$) but the number of rats with tumours in any one weight group was small.

Although only 19 of the 220 females had fibrosarcomas, examination of weight at the eight age intervals showed a significant relationship with weight at 78 wk of age and with the weight ratios at 54 and 78 wk of age ($P < 0.05$). No significant relationships between weight and tumour incidence was seen among males with a fibrosarcoma.

Pancreatic islet-cell tumours

Islet-cell tumours were seen in 31 males and there was a significant correlation with body weight at the age of 30 and 54 wk ($P < 0.05$ and $P < 0.01$, respectively) but not with early body weight. The weight ratio at wk 54 was also significantly correlated ($P < 0.05$).

Other tumours

Tumours of the adrenal medulla were seen in 17 males and no association with body weight was evident in the analysis for body weight at successive ages. There were 21 CD males and eight CFY males with lipomatous tumours and the combined data for males showed a significant trend in the body weight gain at 54 wk of age ($P < 0.05$) and at each of the eight age points the observed incidence in the heavy group exceeded the expected value. In the CD males there was a significant relationship with body weight at 10 and 54 wk of age ($P < 0.05$). No such relationship among CFY males was detected.

Fatal and incidental tumours

It is not always easy to decide whether a particular neoplasm caused, or significantly contributed to, death, particularly where an animal is also suffering from a debilitating non-neoplastic disease. For the analyses reported in Table 4 we accepted the opinion of the pathologists who classified tumours as incidental or fatal. However, since the assumption that one rat can die of two causes—a tumour and severe

Table 5. Significance of relationships between body weight or weight ratio and the number of animals with tumours of various types, after assigning tumours in rats with marked or severe glomerulonephrosis as incidental

		Level of significance†			
	Tumour...	Pituitary		Mammary	Fibromatous
Age (wk)	Sex...	Males	Females	Females	Females
		Body weight			
10			**	***	
54		*	**	***	
		Weight ratio§			
54/5		(*)	*	***	**

†See Methods section for details of statistical analysis. Trend statistic: (*)$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.
‡Excluding mammary gland fibroma.
§Ratio between weight at 54 wk of age and weight at 5 wk.

progressive nephropathy—may not be reasonable, a selective reanalysis was made after reclassifying as incidental those tumours that occurred in rats with marked or severe progressive nephropathy. For this reanalysis (see Table 5) only the 10- and 54-wk weights and the 54-wk weight ratios were used as they were considered representative. Only fibromatous, pituitary and mammary tumours were studied as they showed the strongest association with body weight.

As expected, the strength of the association between body weight and tumour incidence was reduced from that in Table 4 by this reclassification. However, many of the specific associations re-examined remained highly significant.

DISCUSSION

A strong relationship between body weight and time of death was seen. This underlined the necessity for taking age and death into account in the analyses relating body weight to tumour incidence, as the number of rats in the heavier groups surviving to ages where tumours were more common was markedly less than the numbers in the lighter groups.

After standardizing for age at death, the association between heavy body weight and the development of certain types of tumours was striking. The statistical significance of the relationship is probably underestimated if the death of animals in which severe progressive nephropathy and tumours coexist is always attributed to nephropathy rather than to a coexisting tumour, as for Table 5. However, adherence to the pathologists' opinions on the cause of death, as for Table 4, probably overestimated the strength of the relationship slightly. The overall conclusion is that among groups of control Sprague–Dawley (CFY and CD strain) rats in two typical chronic toxicity tests, some animals attained a relatively high body weight and then had an increased risk of progressive nephropathy and tumour formation. These heavy animals consequently had relatively poor survival.

The animals that gained most weight may simply have eaten more than other animals in the cages. There was a correlation between food conversion efficiency, mature body weight and tumour occurrence in the male rats studied by Ross *et al.* (1982 & 1983a,b). Any correlation involving food conversion efficiency in the control animals in this study would not have been detected since animals were not individually housed. The range of body weights of supposedly similar rats in control groups in typical chronic toxicity tests is an expression of an uncontrolled and unknown variable within the study design. The finding that mortality together with incidence of progressive nephropathy and tumours is highest in the heavy animals is, therefore, of some concern.

The same variability in individual body weight, and the correlation of body weight with tumour incidence, is likely to be present in the groups of rats given the test chemical in toxicity studies. An effect of the chemical on the range of individual body weights may indirectly alter survival and tumour incidence. If the test chemical is given in the animals' feed and makes it less palatable or if there are changes in the food conversion efficiency, some difference in survival and tumour frequency would appear likely, compared with the concurrent control groups. Within 25 feeding studies in the US National Cancer Institute programme there was no apparent association between survival and reduced body-weight gain among treated F344 rats, but survival was generally greater in the treated groups compared with controls. The overall incidence of several types of tumours in the treated rats at the high-dose levels in these studies was lower than in the controls, including pituitary tumours in males (17.4% and 19.4%, respectively) and females (41.6% and 46.6%, respectively), mammary fibroadenomas in females (16.5% and 24.8%, respectively) and pancreatic islet-cell tumours in males (3.9% and 4.9%, respectively) (Haseman, 1983). These apparent decreases may not have been chance alone since these were the types of tumours that were affected by body weight in Sprague–Dawley CD and CFY strain rats in the present study.

Decreased incidences of certain tumours and changes in survival in tests for carcinogenicity of chemical substances suggest that non-specific factors are influencing important parameters in the studies. These non-specific factors may exaggerate or obscure the chemical-specific effects on tumour incidence, yielding false positive or false negative determinations for carcinogenic potential (Conybeare,

1980). Variability in body weight and the possible effect of this on the outcome of studies are at least partly avoidable. Precise information on the age of animals and narrow limits for acceptable body weight at weaning and in the following few weeks would be one approach. Manipulation of the diet composition or availability, to limit the growth of the individuals with the highest potential body weight, also seems advisable.

Current protocols for chronic toxicity studies in rodents require the pathologist to distinguish between lesions contributing to death and incidental lesions. In view of the correlation between body weight and kidney progressive nephropathy, leading to poor survival, there is clearly a particular need to distinguish correctly between incidental and fatal lesions. Any bias in attributing death to a tumour, rather than to coexisting severe progressive nephropathy, increases the likelihood of non-specific effects on body weight affecting the analysis of tumour incidence. Single-variable analyses of tumour incidence can be misleading (Ross *et al.* 1982). In chronic toxicity studies with marginally increased or decreased incidences of certain tumours in treated groups there is possible benefit from extending the examination of individual animal data on body weight and pathology, particularly if the tumour incidence falls within the range encountered historically in control groups in the same laboratory or in other laboratories.

The results of the analyses reported in this paper raise a question about the interpretation of chronic toxicity and carcinogenicity studies. Since weight gain non-specifically influences survival and tumour risk, should it be regarded as a variable to be routinely controlled for in the evaluation of the results of chronic toxicity and carcinogenicity studies in rats?

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