Human Risk Assessment— The Role of Animal Selection and Extrapolation

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Opinions on animal selection for the assessment of carcinogenicity

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I had a bad dream. I dreamed that I was dead. But that wasn't really the bad part. The obituary notices were really quite good. They said such things as "He was industrious", "He was thoughtful and imaginative in his opinions", "He was a prolific contributor to the scientific literature but arguably contributed too much to conference proceedings which no one read", and so on. No, it wasn't the being dead or the obituaries that were the bad aspect of the dream, it was the following interview with Himself-up-Above (HUA) that was so disturbing!

HUA. So you practised as a toxicologist?

ME. Yes Sir.

HUA. In the process you took some of My creatures out of the wild. You confined them to small boxes and you deliberately encouraged them to mate in such a way that diseases which had not been eliminated by My masterly 'Evolution–Natural Selection Scheme' were not only perpetuated but actually fostered. Can you tell Me – why did you do these things?

ME. Please Sir, I thought that the main object of your 'Evolutionary–Natural Selection Scheme' was to evolve Man (in the image of your Goodself). The aim of we toxicologists was to try to prevent disease in Man, and we thought You would like this.

HUA. It was presumptuous of you to think you knew the object of My Scheme. But why were you so unbelievably naive and stupid in what you actually did?

ME. Please Sir, may I sit down, I feel a bit faint?

HUA. In your experience as a toxicologist did you ever encounter a rat or mouse who felt faint?

ME. I do not know, Sir. They can't speak – only squeak.

HUA. If that is a criticism of one of My Creations, don't be impertinent! I'll ask you a related question. Did you ever encounter a rat or mouse that died from coronary thrombosis?

ME. No Sir.

HUA. You knew very well that your fellow Men were committing suicide in large numbers by eating some of My other Creations in excess and developing cardio-vascular disease. You knew that this was the most common cause of premature death among your fellow Men. Nevertheless, you quite deliberately chose two species, rats and mice, for testing new chemicals to see if they might endanger man's health.

ME. But our main objective was to try to prevent man from developing cancers.

HUA. If that was your aim, why did you conduct your experiments under conditions that were so unnatural and which actually predisposed them to develop cancers in

Table 3. Proportions of cancer deaths attributed to various different factors.

Factor or class of factors	Percentage of all cancer deaths	
	Best estimate	Range of acceptable estimates
Tobacco	30	25–40
Alcohol	3	2–4
Diet	35	10–70
Food additives	<1	−5†–2
Reproductive and sexual behaviour	7	1–13
Occupation	4	2–8
Pollution	2	<1–5
Industrial products	<1	<1–2
Medicines and medical procedures	1	0·5–3
Geophysical factors	3	2–4
Infection	10?	1–?
Unknown	?	?

From Doll & Peto (1981).
† Allowing for a possibly protective effect of antioxidants and other preservatives.

geophysical factors and infection probably account for nearly 9 out of every 10 deaths from cancer among humans (Table 3). By contrast, they estimated that fewer than 1% are attributable to food additives (indeed, the addition of some chemicals to food may actually reduce cancer risk). Their best estimate for the contribution of occupational factors was 4% and for that of medicines and medical procedures, 1%. Industrial products chipped in with less than 1% and pollution with 2%.

Of course, one may argue that the low estimates for food additives and medicines, etc., reflect the effectiveness of existing test requirements. Even so, we are left with 30% of human cancer attributable to smoking, for which there is no obvious animal model, and the huge total of 35% for general dietary factors which remains largely ill-defined and uninvestigated. Until we have a much better idea of which dietary factors are important determinants of cancer risk in man, we have no basis for believing that one animal model is superior to any other.

Selection of species for carcinogenicity testing

At many meetings someone or other expresses the view that the ideal animal for use for the carcinogenicity testing of a compound is the one that metabolizes it in the same way as man. Whatever the theoretical merits of this

view, in practice it rarely has much value. First, it assumes that there actually exists a species which mimics man in the way it metabolizes the particular compound. Secondly, it ignores the possibility that although the metabolism may be similar, the distribution of some important receptor site may be different. Thirdly, it overlooks the fact that it could be more expensive to identify a species that mimics man than to carry out a carcinogenicity test in a rodent. Finally, it ignores the very serious constraint that, for a carcinogenicity test to be meaningful, it must be conducted in a sufficiently large number of animals to permit a statistically significant effect on cancer risk to be seen. Also, before carcinogenicity activity can be excluded with any degree of confidence, animals must have been exposed to the test agent for the majority of their natural lifespan. If, by chance, the Marion's tortoise (*Testudo sumeirii*) turned out to be the one species that handled a chemical in the same way as man, it would be for toxicologists in one's grandchildren's generation to evaluate the results of a study started now. From the viewpoint of timing, it would be easier to assess whether a chemical is safe for giant tortoises by testing it in man, than vice versa!

In practice, therefore, for logistic reasons, only a very limited number of species (rats, mice, hamsters and possibly dogs) can be used for routine carcinogenicity testing irrespective of whether they metabolize compounds in the same way man does.

Which strain? Inbred or outbred?

The choice of strain for a carcinogenicity test is heavily dependent on the precise aim of the study. If the main aim is simply to obtain reproducible results irrespective of what they may mean, then unquestionably one should choose an inbred strain or an F_1 hybrid. However, even if one does this, reproducibility is not always very good, particularly between laboratories. Environmental variables, particularly diet, but also other variables that have not been clearly defined, influence the incidence of spontaneously arising neoplasms to a major extent.

Alternatively, if the main aim of a carcinogenicity test is, as it should surely be, to provide data which is useful in the prediction of *possible cancer risk in man* or, equally importantly, *likely freedom from cancer risk in man*, then the emphasis of choice should be on avoiding the use of strains of animals which are genetically flawed in such a way that they develop 'spontaneously' very high incidences of tumours of kinds which are rare, or do not occur at all, in man. The high incidences of testicular, pituitary and mammary tumours in many strains of rats and the high incidences of liver, lung and lympho-reticular neoplasms in many strains of mice are, in my view, serious handicaps to meaningful predictive carcinogenicity testing. The extent to which these

Table 4. Reported incidence of adrenal medullary tumours in three different strains of male and female rats

Strain	Tumour development (%) in untreated rats		Reference
	Males	Females	
Wistar-derived	81	56	Gillman *et al.* (1953)
	0	2	Boorman & Hollander (1972)
Sprague–Dawley	51	8	Kociba *et al.* (1979)
	16	4	Thompson & Hunt (1963)
Fischer 344	37	12	Jacobs & Huseby (1967)
	4	0·5	Sass *et al.* (1975)

characteristics represent genetic flaws and the extent to which they reflect overfeeding and inappropriate environmental conditions is presently uncertain, although it is already clear that background tumour incidence in the long-term rodent studies can be greatly reduced by the avoidance of overfeeding.

When considering the reality of the present situation, I am far from happy that either the Fischer 344 rat or the B6C3F1 hybrid mouse are really suitable for predicting cancer risk or lack of cancer risk for man. However, I know of no other strains, either inbred or outbred, that are more suitable.

Table 4 illustrates the variation in incidence of adrenal medullary tumours in three strains of rats – two random-bred (Wistar and Sprague–Dawley) and one inbred (Fischer 344) – in long-term studies. Clearly, it would be meaningless to regard any of these strains as especially prone or especially resistant to the 'spontaneous' development of adrenal medullary tumours. On the other hand, it is clear that dietary composition may greatly influence the incidence of these tumours in rats (see Table 5).

During recent years, I have come to realize that apparent differences in response between different strains of rats (or different strains of mice) to the same chemical agent are more likely to be due to differences between the diets fed to the animals or to other differences in laboratory environments than to

Table 5. Effect of composition of diet on incidence of adrenal medullary tumours in rats.

Diet composition (%)			Life-time incidence of adrenal medullary tumour (%)*	
Carbohydrate	Protein	Fat	Males	Females
60	15	11	63	47
4	82	10	13	15

From Gilbert *et al.* (1958).

Table 6. Endocrine tumour incidence in control rats (%) and significant effects of exposure (↑ or ↓) to the same neuroleptic drug in three separate two year studies of similar design.

	Strain of rat					
	Wistar I		Wistar II		Sprague–Dawley II	
Endocrine tumour	Male	Female	Male	Female	Male	Female
Pituitary	22(↑)	62	17	53	41	46
Mammary						
Benign	0	86	2	11	8	77
Malignant	0(↑)	10(↑)	0	0	2(↑)	22
Adrenal						
Medulla	18(↑)	22	0	0	2	0
Cortex	10	10	0	0	0	3
Thymoma (endocrine type)	4(↑)	10(↑)	0	0	0	0
Thyroid						
Follicular	26(↓)	18	0	0	0	0
C-cell	0	6	9	5	1	0
Pancreas						
Islet cell	4(↑)	0(↑)	4(↑)	3(↑)	4	5

I and II, indicates laboratory experiment carried out in.

genetic differences between the strains. A striking example of this is illustrated in Table 6, in which the results of three very similar two-year studies on the same neuroleptic drug gave rise to three very different results. In Sprague–Dawley rats, the only statistically significant effect was an increased incidence of mammary tumours. In one study in Wistar rats at the same laboratory, the only effect was an increased incidence of insulinomas in both sexes. But in another study in Wistar rats in a different laboratory, increased incidences of mammary, pancreatic islet cell and thymic (endocrine-type) tumours were seen in both sexes, and increased adrenal medullary and pituitary tumours and decreased thyroid follicular tumours were seen in males. In the light of such variation in response, between nominally the same strain of rat under different conditions and between different strains of rat under the same conditions, I feel that great caution is necessary in attributing apparent differences in response solely to genetic constitution.

Extrapolation to man

Extrapolation is a mathematical term referring to the calculation from known terms of a series of other terms. Its use by toxicologists to bridge the gap between rodent and man is, to say the least, etymologically dubious. Indeed, it is almost beyond belief that toxicologists uncomplainingly allowed, under the

Table 7. Gaps to be bridged in extrapolating results from laboratory rodents to man.

Differences in
1. Body size, basic metabolic rate and longevity
2. Extent of inbreeding
3. Composition of, and day-to-day variation in diet; Coprophagia.
4. Indulgence in alcohol, tobacco, contraceptive pill and drugs
5. Exercise
6. Opportunity for sexual fulfilment.
7. Spectra of commonly occuring diseases and common causes of death
8. Speech: ability to describe symptoms and availability of surgery and other forms of therapy
9. Information available on morbidity
10. Information available on cause of death and incidental findings at death

umbrella term 'extrapolation', their findings in carefully conducted laboratory studies to be manipulated by statisticians and translated into risk assessments for man. Table 7 lists some of the gaps which such extrapolations ignore. Surely common sense dictates that if one cannot predict from the results in one strain of rat what will happen in another strain, or even in the same strain in another laboratory (Table 6), how can one hope to predict across the rodent–man species gap and across the other gaps listed in Table 7?

Effects of overfeeding on non-neoplastic disease in rats

Although several decades have passed since Tannenbaum and Silverstone began to report the effects of caloric intake and dietary composition on tumour incidence in rats and mice (for review see Clayson, 1975), and despite

Table 8. The effects of overfeeding on the incidence of certain non-neoplastic diseases in untreated male Sprague–Dawley rats

Disease	Percentage of sample developing disease
Moderate to severe glomeronephritis	67
Parathyroid hyperplasia	67
Calcification of aorta	34
Adrenal medullary	
hyperplasia/neoplasia	32
neoplasia	20
Chronic fibrosing myocarditis	83

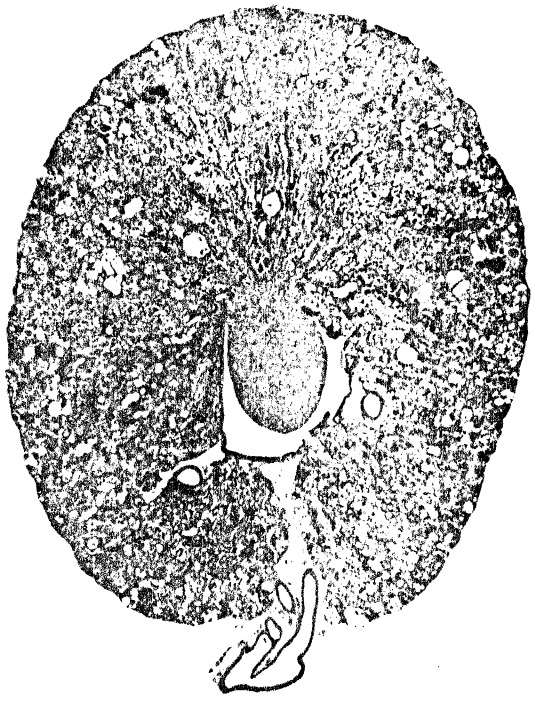

Figure 1. Photomicrograph of chronic progressive nephropathy in untreated male Wistar rats given free access 24 h each day to a standard laboratory diet for a period of 2 years.

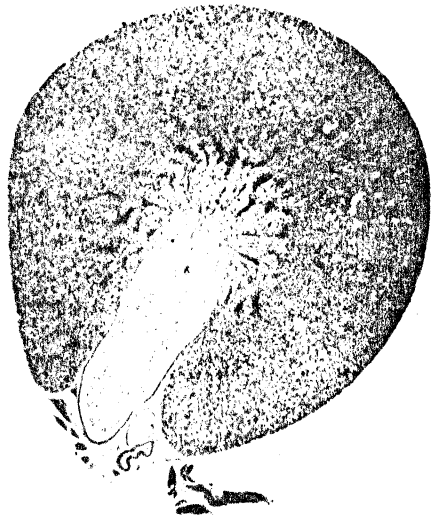

Figure 2. Photomicrograph of the kidney in untreated male Wistar rats given access to a standard laboratory diet for six hours per day for two years.

numerous publications by myself and others during more recent years (Roe & Tucker, 1973; Tucker, 1979; Conybeare, 1980; Roe, 1981), the folly of overfeeding animals during the conduct of carcinogenicity studies persists. Table 8 illustrates some of the dire consequences in terms of the incidences of certain non-neoplastic diseases resulting from overfeeding in untreated male Sprague–Dawley rats which I encountered in a recent study. Figures 1 and 2 illustrate how overfeeding affects the severity of chronic progressive nephropathy in untreated male Wistar rats. Figure 1 was prepared from an animal given free access throughout the 24 h of each day to a standard laboratory diet for a period of 2 years. By comparison, Figure 2 was prepared from an exactly comparable rat that was given access to the same diet but for only 6 h per day for 2 years. Chronic progressive nephropathy may lead to a severe disturbance of calcium homeostasis, with consequent parathyroid hyperplasia, and cortical nephrocalcinosis which parathyroid hyperplasia gives rise to in kidneys already severely affected by progressive nephropathy.

Table 9. Association of metastatic calcification of aorta, lung and kidney with adrenal medullary hyperplasia and/or neoplasia in animals in a two year carcinogenic study.

		Adrenal medullary hyperplasia/neoplasia	
		−	+
Metastatic calcification (aorta, kidney, lung, etc.)			
	−	55	26
	+	14	24

Significance of positive association: $P = 0{\cdot}01$.

Figure 3. Effects of overfeeding on adrenal medullary tumour incidence.

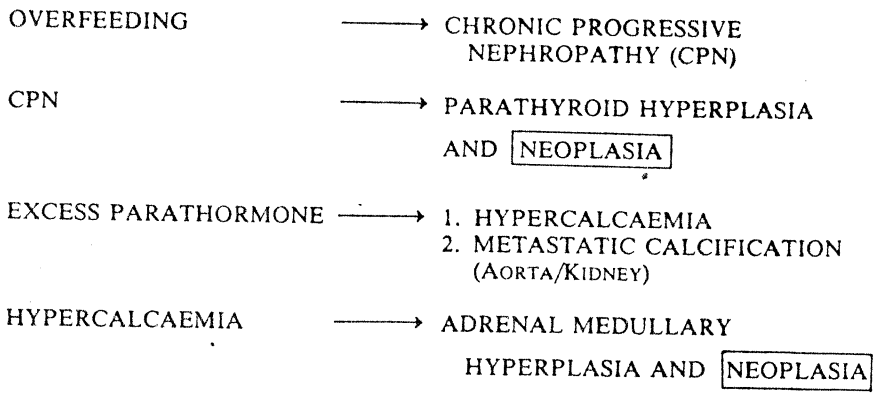

Table 9 shows the statistically significant ($P < 0{\cdot}01$) association that occurs between metastatic calcification of the aorta, kidney, lung, etc. and adrenal medullary hyperplasia and/or neoplasia in the two-year carcinogenicity study illustrated in Table 8. Figure 3 illustrates a sequence of effects linking overfeeding to increased adrenal medullary tumour incidence.

Effects of high concentration of polyols or lactose in the diet of rats

During recent years, there has been concern that certain polyols, including sorbitol, xylitol and lactitol, when fed in high dietary concentrations to rats, predispose to adrenal medullary hyperplasia and neoplasia (Roe & Baer, 1985). The clue to the mechanism involved came from observations on the long-term effects of high dietary concentrations of lactose. Like the polyols, lactose increases the absorption of calcium from the gut of rats. This increased calcium absorption is associated with pelvic nephrocalcinosis and with adrenal medullary hyperplasia and neoplasia. Table 10 summarizes the data in terms of the effect of 20% dietary lactose on the incidence of adrenal proliferative changes.

Table 10. Effect of 20% dietary lactose on adrenal medulla in rats.

	Rats with tumours (%)			
	Males		Females	
Adrenal medullary tumours	Control	20% Lactose	Control	20% Lactose
Hyperplasia or phaeochromocytoma	41	71	16	26
Phaeochromocytoma	23	44	2	4
Malignant phaeochromocytoma	7	20	0	2

Effects of overfeeding on incidence of neoplastic disease

Previously, much attention (Roe, 1981) has been drawn to the outrageously high incidence of neoplasia which has come to be accepted as the norm for control groups in carcinogenicity studies on rats. To illustrate this, Table 11 depicts the incidences of certain kinds of neoplasia in the untreated Sprague–Dawley rats which constituted the controls in a definitive carcinogenicity study on 2,4,5-T (Kociba *et al.*, 1979). Table 12 illustrates how simple dietary restriction can dramatically reduce two of the kinds of tumour listed in the previous tables, namely, tumours of the pituitary and mammary gland. Table 13 illustrates how dietary restriction can reduce the incidence of tumours of many kinds in mice, including lung, liver and lympho-reticular.

Table 11. Hormone-associated neoplasms (%) in *ad libitum* fed untreated control Sprague–Dawley rats observed for up to 26 months (86 rats of each sex).

Site/type of neoplasm	Rats with neoplasms (%)	
	Males	Females
Pituitary	31	63
Adrenal		
Cortex	2	7
Medulla	51	8
Thyroid		
C-cell	8	8
Parathyroid	0	1
Pancreas		
Exocrine	33	0
Endocrine	16	9
Testis	7	—
Ovary	—	5
Mammary gland	—	
Fibroadenoma		76
Adenoma	5	12
Other	—	29

From Kociba *et al.* (1979).

Table 12. Effect of dietary restriction on incidence of pituitary and mammary tumours in rats.

Tumour	% Rats with tumours under different feeding regimens			
	Males		Females	
	Ad lib.	Restricted	*Ad lib.*	Restricted
Pituitary	3[2]	0***	66	39**
Mammary	0	0	34	6***

From Tucker (1979).
**$P < 0{\cdot}01$, ***$P < 0{\cdot}001$.

Concluding remarks

For many years I have been drawing attention to the need for basic research designed to define the conditions needed for the maintenance of laboratory rats and mice in good health until they are old. So far, my pleas have seemingly fallen mainly on deaf ears, although some research in this area has now been started or is planned. In rats, overfeeding predisposes to all manner of endo-

Table 13. Effect of simple dietary restriction on tumour incidence in mice.

Tumour	Mice (no.) developing tumours at any time during study†			
	Males		Females	
	Ad. lib	Restricted to 75% of *ad lib.*	*Ad lib.*	Restricted to 75% of *ad lib.*
Lung	30	19*	24	8**
Liver	47	12***	7	1*
Lymphoma	4	1	11	4*
Other	8	4	12	4*
Any tumour at any site	71	36***	50	17**
Any malignant tumour	17	7*	23	7**

From Conybeare (1980).
† $n = 160$ males, 160 females.
* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

crine disturbances and these are bound to distort the response of animals exposed to chemicals in carcinogenicity tests. There can be no sense in testing chemicals for carcinogenicity in rats maintained under conditions such that 50–100% of them develop pituitary and mammary tumours, etc. There is no identifiable population of humans for which such rats could constitute a model.

I have no doubt that many of the findings in carcinogenicity studies carried out in overfed rats and mice are no more than nonsensical gobbledygook. The problem is that where these effects suggest a beneficial effect of treatment on the incidence of a particular type of tumour, Regulatory Authorities ignore them, whereas adverse effects are regarded as evidence of carcinogenicity. Elsewhere (Roe, 1983), I have proposed the term 'pseudocarcinogenicity' to describe the enhancement of tumour risk by a non-genotoxic mechanism in animals plagued with abnormalities because of overfeeding and laboratory-associated artefacts.

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