Animals and Alternatives in Toxicity Testing

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Carcinogenicity Testing

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I need first to make clear that I fully associate myself with the aims of FRAME and greatly admire the efforts they are making, especially the organisation of this Meeting. However, I find that the sections of the FRAME Toxicity Committee's Report on long-term toxicity testing and on carcinogenicity testing inadequately recognise the extent to which present methods of testing are unsatisfactory. During the last few years I have been saying increasingly loudly that the way we keep and overfeed laboratory animals renders them exceedingly prone to laboratory artefacts, including very high incidences of neoplasms of various kinds and numerous manifestations of endocrine disturbance. These artefacts interfere with the interpretation of tests and frequently lead to their having to be repeated.

I disagree with conclusion 8 in the Summary of Conclusions and Recommendations section of the Report, "Except when carcinogenicity is being investigated, or where there is good scientific evidence to suggest to the contrary, the routine long-term toxicity test should not exceed 6 months duration". I am aware of many examples where non-predictable toxic effects have not become manifest in rats or mice until much later than this. A test which goes on for only 6 months covers only the rapid growth period of life plus a short part of early adult life, and would be irrelevant to the detection of effects on diseases which arise late in life or are the consequence of cumulative toxicity. It is sometimes possible to reduce the numbers of animals used by combining chronic toxicity testing with lifespan carcinogenicity testing and this is commonly done. In any case, it is in my view indefensible not to look as hard as possible for non-neoplastic pathological affects as well as neoplasms in the evaluation of carcinogenicity tests.

The Preliminary Report does not address adequately the distinction between carcinogens which initiate cancer by interacting with DNA and those which predispose to cancer by one of a wide variety of other mechanisms. In the present state of our knowledge, there are some grounds for hoping that in vitro

methods might eventually be reliable enough largely to replace animal tests for the detection of genotoxic carcinogenicity. But the subject of non-genetic carcinogenesis is still in its infancy. All we know is that a wide variety of quite different mechanisms can be involved and that many of these entail the disturbance of homeostasis at the tissue level or whole animal level rather than at the cellular level. It is, therefore, in my view, premature to suggest that the search for in vitro methods for detecting non-genotoxic carcinogenicity should be encouraged.

In August of this year the DHSS published its "Guidelines for the Testing of Chemicals for Carcinogenicity", and I commend this document to all who are involved in the problem of carcinogenicity testing as the wisest official document on this subject so far published.

There are at this Meeting many notable experts on carcinogenicity, but only a few of them spend many hours every week looking down a microscope at sections of tissues from animals in carcinogenicity tests. It is one thing to think theoretically about carcinogenesis mechanisms, it is another to look at tumour incidence tables for significant differences between treated and control groups, and yet another still actually to look at the living animals and at their tissues after necropsy. Let me share this experience with you.

1. Here is a list of per cent incidences of certain lesions in control Sprague-Dawley male rats in a carcinogenicity study which I am presently reading:

Moderate to severe glomeronephritis	67
Parathyroid hyperplasia	67
Calcification of aorta	34
Adrenal medullary - hyperplasia/neoplasia	32
- neoplasia	20
Chronic fibrosing myocarditis	83

Does anyone know a human population for which these untreated rats would be an obvious model? These animals would, on the basis of these figures, be useless for assessing renal toxicity, cardiovascular toxicity, and disturbance of calcium metabolism.

2. The following hormone-associated neoplasms (%) were found by Kociba et al. (1) in ad libitum fed Sprague-Dawley rats (86 of each sex) observed for up to 26 months:

		Male	Female
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

Could one have any confidence that an experiment conducted in such rats would be of any value for detecting a carcinogen acting on the female breast, or on the pancreas? In such rats one does not think in terms of percentage of animals with cancer, but in terms of mean numbers of, say, 3-10 tumours per rat.

3. At least six unnatural aspects of a laboratory rat's life clearly predispose it to a wide variety of disturbances of endocrine status, including the development of benign and malignant tumours of endocrine glands and of hormone-influenced tissues:

Food available 24 hours per day
Excessively nutritious diet
No need to forage
No need to avoid predators
Enforced celibacy despite sexual stimulation
General boredom

4. Consider the serum prolactin levels (ng/ml) in ad libitum fed Sprague-Dawley rats:

Age (months)	Male	Female
2	26	21
3	27	37
4	28	34
7	35	74
13	128	214
19	119	345

These and even higher figures in other experiments are secondary to hyperplasia and neoplasia of the pituitary gland - the tumours being prolactinomas. (N.B. The prolactin level in non-pregnant women is 20-40 ng/ml.)

5. The work of Tucker (2) illustrates the beneficial effect of controlled feeding, as distinct from ad libitum feeding, on the incidence of pituitary and mammary tumours:

	Male		Female	
Feeding regimen	ad lib.	restricted	ad lib.	restricted
Rats with pituitary tumours (%)	32	0	66	39
Rats with mammary tumours (%)	0	0	34	6

Conybeare (3), in an experiment involving groups of 160 male and 160 female mice, found that "controlled" feeding, as distinct from "uncontrolled", ad libitum feeding, reduced the incidences of a wide variety of tumours, including many of a non-endocrine nature:

	Male		Female	
Feeding regimen	ad lib.	75% of ad lib.	ad lib.	75% of ad lib.
Type of tumour				
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

6. Dietary fat can reduce liver tumour incidence dramatically in C57BL female mice (4):

	Mice with liver tumours (%)	
	Benign or malignant	Malignant
SS diet with 5% GNO	8	1
SS diet with 10% GNO	43 9	

(GNO = groundnut oil)

My plea is that if we want to cut down the numbers of animals used in long-term toxicity and carcinogencity testing, then we should fund and undertake research aimed at defining the conditions in which untreated laboratory animals can be maintained in good health until they are old. The research most needed relates to reducing the present astronomically high incidences of endocrine disturbances and tumours. Better control of the quality and quantity of diet is probably more than half the battle, but other aspects of animal husbandry need attention, particularly sexual frustration. In the long run, research of this kind will reduce the unnecessary use of animals by far more than pious hopes based on misconceptions of what carcinogenicity is all about.

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New Approaches

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The so-called "classic" procedures for the testing of potential carcinogens in animals have not changed fundamentally for the