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# Assessment of Inhalation Hazards

## Integration and Extrapolation Using Diverse Data



Springer-Verlag Berlin Heidelberg New York
London Paris Tokyo Hong Kong

# 6. The Quality and Relevance of Data from Studies in Laboratory Rodents

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## The Rodent as a Model for Predicting Chronic Toxicity and Enhancement of Ageing-Related Diseases in Humans

The rationale for conducting toxicity and carcinogenicity tests of chemicals in laboratory animals assumes that the information obtained will be useful in the prediction of how humans will respond to exposure to the same chemicals. This assumption is reasonably well based in terms of response to short-term high-dose exposure to chemicals, but much less so in terms of later responses to lower doses. As animals grow older, it becomes more and more difficult to distinguish between toxic effects and changes attributable to ageing. Indeed, in many long-term experiments in rodents, most of the differences between exposed and control groups are simply in the incidence and severity of ageing-related diseases. Since the spectra of the most common ageing-related diseases which afflict humans and laboratory rodents are quite different, it is only to be expected that the actual manifestations of chronic toxicity in rodents are quite different from those to be expected in humans.

It is nowadays generally accepted as reasonable to distinguish between *genotoxic* and *non-genotoxic* mechanisms in carcinogenesis (Butterworth and Slaga 1987; Roe 1988a). In the case of the former, there is often a close similarity between the responses of different species. Furthermore, where differences occur, they can sometimes be explained by differences in metabolism and/or distribution of cells with detoxifying or metabolically activating enzymes. By contrast, non-genotoxic mechanisms—of which many are known and doubtless many more are awaiting discovery—are often seemingly species specific. Such mechanisms often involve disturbances of endocrine or other homeostatic control systems, and in many cases the effects of exposure to non-genotoxic carcinogens seen in the late stages of carcinogenicity tests seem to be simply enhancements of spontaneously arising, ageing-related neoplasia. It has long been recognized that, in rodent studies on chemicals, it is easier to enhance the

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incidence of a commonly occurring tumour than that of a rare tumour. It may well be that this is because the underlying disturbances of physiological and endocrine status involved in non-genotoxic carcinogenesis are ageing related. In other words, conditions which either specifically or non-specifically cause ageing will *pari passu* enhance the risk of non-genotoxic carcinogenicity.

In any event, the important points for epidemiologists to appreciate are: (a) *that many manifestations of chronic toxicity relate more to the ways in which ageing affects a species than to the nature of chemicals which predispose to premature ageing*; and (b) *that there can be no mathematical formula for predicting non-genotoxic carcinogenicity for humans from the results of laboratory tests.*

## Influence of Caloric Intake on Ageing-Related Disease and Neoplasia in Rodents

The amount of food a laboratory rat or mouse consumes during a carcinogenicity test on a chemical may have a highly significant influence on how long it lives and on the age-standardised incidence of ageing-related and neoplastic lesions (Conybeare 1988). In the past, far too little attention has been paid by experimentalists to this fact, and it is not surprising, therefore, that many statisticians extrapolating from animal data to humans have totally ignored it. Experimentalists tend to argue that, since test and control animals are offered the same diet, one is able to compare "like" with "like." However, if for one reason or another the test chemical increases or reduces the food intake of animals, then one ends up being unable to compare like with like. Epidemiologists argue that humans also overeat and are therefore like overfed rats. However, this is not strictly true. Humans are really not like rodents, confined to a small cage and given nothing else to do but eat a diet which nutritionally far exceeds their needs. In the case of rats, the manifestations of overnutrition include severe, and sometimes fatal, renal disease and many kinds of endocrine disturbance (see Table 6.1). Thus, one may encounter almost 100% incidences of pituitary, mammary and interstitial cell tumours of the testis in control as well as in test groups.

In a recently briefly reported study (Roe 1988b), my colleagues and I reported that the incidence of life-threatening, or actually fatal, malignant neoplasms in rats was significantly higher in rats given access to food throughout the 24 h of each day than in rats offered the same diet but for only 6.5 h per day. Common sense dictates that one should regard findings in animals rendered grossly abnormal because of overfeeding as very dubious predictors of chronic toxicity in humans.

Doubtless, many humans also consume more calories than they need, and there is good evidence that this predisposes to premature death and earlier onset of life-threatening diseases including some forms of cancer. However, the effects of overnutrition in rats on the incidence of the diseases listed in Table 6.1 seemingly have little or no parallel in humans.

**Table 6.1.** Overnutrition-related diseases in laboratory rats.

| |
|---|
| Non-neoplastic |
| Chronic progressive nephropathy |
| Cortico-medullary and pelvic nephrocalcinosis |
| Mesenteric and pancreatic hypertrophic periarteritis |
| Radiculo-neuropathy affecting the cauda equina |
| Inflammatory skin lesions |
| Parathyroid hyperplasia |
| Widespread metastatic calcification |
| Neoplastic |
| Benign and malignant tumors of |
| Pituitary |
| Mammary gland |
| Adrenal medulla |
| Leydig cells of testis |
| C-cells of thyroid |
| Islet cells of pancreas |

## Route of Exposure and Dose

Before the distinction between genotoxic and non-genotoxic carcinogenicity began to be understood, a positive result in a carcinogenicity test was taken to indicate that the agent was "intrinsically" carcinogenic irrespective of the route or dose of administration. We now know somewhat better. Sarcoma induction in a rat following the subcutaneous injection of a chemical to which humans are only exposed by the inhalation route would not nowadays be accepted as adequate evidence of carcinogenic risk for humans since, although the cancer may have arisen because the test substance is a genotoxic carcinogen, there is also a big chance that a non-specific mechanism is responsible (Grasso and Golberg 1966).

However, there remain areas of uncertainty. The relevance of intratracheal instillation and intrapleural or intraperitoneal injection in rodents for distinguishing carcinogenic from non-carcinogenic inhalable dusts is a subject for debate among investigators. The sheer inelegance of these methods for exposure offends those who are concerned with the importance of dose at the tissue and cellular level. Also, increasing evidence of lack of correlation between (a) the results of tests based on these methods of exposure; (b) the results of tests involving exposure by inhalation; and (c) epidemiological data suggests that, while such tests may have a place in screening for possible carcinogenicity, they are wholly unreliable for quantitative risk assessment.

The science and technology of inhalation toxicity have advanced considerably during the last 2 decades. Nevertheless, there is one obstacle to the development of realistic animal models which is seemingly insuperable. Despite the strong epidemiological evidence of an association between cigarette smoking and lung cancer risk in humans, there is no acceptable animal model for this. Three factors contribute to the problem. First, when rats and mice are exposed to cigarette

smoke, it is via the nose and not, as in smokers, via the mouth. Second, a heavy smoker may expose him- or herself off or on throughout 16 h or more per day, whereas it is difficult and impossibly expensive to expose laboratory animals to smoke for more than a few hours per day. Third, the nicotine and carbon monoxide components of tobacco smoke are acutely toxic. The human smoker can avoid overdosing by stopping smoking as soon as he or she feels symptoms indicative of toxicity, but the animal under experiment cannot do this. For these reasons, it is not possible to expose laboratory rodents to doses equal to or above those achieved by smokers. Apart from this, one does not really know whether rodents would develop human-type lung cancers even if one could get adequate doses of smoke into their lungs.

The lack of a realistic model for lung carcinogenesis in relation to smoking hampers not only the development of putatively less hazardous cigarettes but also mechanistic studies with inhalable agents to which both smokers and non-smokers may be exposed.

It is in fact interesting to note that, in a study in which rats were exposed to tobacco smoke, the main effect of exposure was not an increase in lung cancer incidence but a reduction in mammary tumour incidence (Davis et al. 1975).

## Extrapolation from High-to-Low Dose

It is not unreasonable to expect to find a positive dose-response relationship for a direct-acting toxin or carcinogen. However, dead cells cannot give rise to clones of cancer cells so that, if the test doses are pushed up to a point where cell death occurs, then the dose-response curve may flatten out or actually fall. In the case of substances which only act as toxins or carcinogens after they have been metabolically activated by tissue enzymes, and in the case of toxins or carcinogens for which the body has only a limited capacity for detoxification, the shape of the dose-response curve may depart from linearity at either end of the dose range. For non-genotoxic agents there can be no general rule whereby one can predict the shape of the dose-response curve. Threshold dose levels below which nothing happens may exist, and only if one has detailed information about how they bring about their observed effects should one presume to be able to extrapolate either upwards or downwards from the dose levels that have actually been studied. Those responsible for making risk assessments should always be mindful of the considerable uncertainties which surround high-to-low dose extrapolation.

## Need for New Techniques for Assessing Disturbances of Physiological Status

Since disturbances of physiological and endocrine status are clearly so important and to some extent antedate manifestations of chronic toxicity and of non-genotoxic carcinogenicity, it would obviously be advantageous to have clinical

techniques for detecting such disturbances and monitoring their severity while animals are still alive. In the rat, since hyperplasia and neoplasia of endocrine tissues are such prominent manifestations of premature ageing and are so commonly associated with overnutrition, it would be very helpful to have methods for measuring the levels of circulating hormones such as prolactin, growth hormone and 17β-oestradiol, etc. in small samples of blood. At present some of the available methods require so much blood that the animal has to be killed for the measurements to be made. Also, assay methods specifically for rat-type hormones as distinct from human-type hormones have not been developed and/or are not generally available.

Thus the depressing picture is that contract research laboratories and other laboratories tend to measure a large number of parameters for which methods are readily available but which are of no more than marginal interest from a toxicological viewpoint, whilst making no measurements on parameters which could provide early evidence of changes indicative of premature ageing and increasing endocrine imbalance.

The development of new methods for measuring such parameters could revolutionise the predictive value of rodent tests. First, one might be able to devise a system of animal husbandry by which rats could be maintained into old age in more or less normal endocrine status. Second, against a background in which untreated control animals remained physiologically normal, one could far more easily detect effects of test substances on endocrine tissues and on the development of ageing-related disease.

## Unrealistic Aspects of Rodent Toxicology

Rodents in the wild differ from laboratory rodents in many ways. Apart from not being confined to a cage, unrestricted sexual activity, a need to forage for food, and a need to avoid predators, wild rodents carry a wide range of parasites and pathogens from which laboratory rats are largely or wholly free. Except in this last particular, the life style of wild rodents is much more like that of humans than is that of laboratory rodents. Although humans are not maintained behind barriers, they are immunized against serious diseases, they are treated for worm and ectoparasitic infestations whenever necessary and they are protected by food hygiene laws. Thus, like laboratory rodents, they are more or less specified pathogen free. The lack of any need to forage for food, the lack of exercise, the lack of opportunity to fulfil sexual urges, the overprovision of unnecessarily nutritious food and general boredom render laboratory rodents exceedingly poor models for humans. Over and above this is another potentially serious and usually unnecessary defect with the model. Rodents commonly eat at night and sleep during the day. Nevertheless, if they are exposed to chemicals other than in the food (e.g. by inhalation or by gavage), such exposure normally takes place during the daytime when they are not eating. Also, blood sampling and other measurements are made during the day. In the case of the safety evaluation of a

drug to which humans are exposed during the day when they are eating, it simply does not make sense to dose an animal by gavage during the daytime when it is not eating. Absorption, metabolism and excretion of ingested test chemicals and many haematological and serum chemistry parameters are influenced by the time of dosing in relation to feeding patterns.

## Tumour Incidence Data in Rodents as a Predictor of Cancer Mortality Data in Humans

Undoubtedly the most serious point that needs to be made concerns the important difference between the data collected by experimentalists conducting carcinogenicity tests in animals and the data collected by cancer epidemiologists. In the laboratory tests, animals are subjected to careful systematic macroscopic examination at necropsy and to routine sectioning of a long list of tissues. The findings are expressed as incidences of benign and malignant tumours in different tissues. Animals which survive to the end of studies may be killed when seemingly quite healthy and yet be found to have one or more small benign tumours in internal organs. Cancer therapy is not offered to rodents in carcinogenicity tests and, for humane reasons, few animals are allowed actually to die from neoplasms. Instead, sick animals are killed. Apart from this, experimentalists are generally very reluctant to diagnose the cause of death in animals that do die. Thus, there are no reliable *cancer mortality* data for laboratory animals in carcinogenicity tests, only *tumor incidence* data.

By contrast, because of general low necropsy rates, epidemiologists have to rely on rather inaccurate cancer mortality data for humans and have virtually no reliable tumour incidence data for internal organs.

A slowly growing benign tumour has a totally different significance from a more rapidly growing, metastasing malignant tumour, and therefore it is simply nonsensical to presume to be able to extrapolate from tumour incidence data in animals to cancer mortality risk in man. Nevertheless, this is presently often done by statisticians making risk assessments.

## Main Conclusions

1. In some ways animals tests for carcinogenicity can provide better data than can be collected by cancer epidemiologists. The effects of high and accurately measured doses can be observed, and pathogenesis and possible mechanisms can be studied because interim sacrifice is considered ethical. However, it is no easier to study the effects of exposure to low doses over long periods in animals than it is in man because of limitations on the numbers of animals that can be studied.

2. The practice of carrying out thorough and systematic necropsies on all animals in carcinogenicity tests provides data that are of great value in the determination of dose-response relationships. However, *cancer mortality* data are not

generated by experimental oncologists – only *tumour incidence* data. Since many of the tumours observed are small, benign and slowly growing, and since comparable tumour incidence data are not available for humans, one cannot make realistic estimates of cancer risk for humans from animal test data.

3. Most carcinogenicity tests in laboratory rats and mice involve *ad libitum* access to overnutritious feedstuffs. Consequently, obesity, premature death, renal disease, polyarteritis, radicular nephropathy and a host of endocrine diseases complicate the interpretation of carcinogenicity studies, particularly in rats. Also, the background incidence of neoplasms in both species is significantly increased by overfeeding such that incidences of lung, liver and lymphoreticular neoplasms may each exceed 30% or even 50% in untreated mice, and pituitary, mammary, adrenal medullary, testicular and other endocrine tumours may approach 100% incidence in untreated rats. Common sense dictates that the results of carcinogenicity tests conducted in such animals cannot possibly be relied upon for the prediction of cancer risks in man.

4. There are many different non-genotoxic mechanisms by which the risk of tumour development may be increased. Many of these mechanisms are seemingly species specific, and many of them entail prolonged disturbance of physiological and/or endocrine functions, and, in the case of some test substances, such disturbances only occur with very high doses. These facts must in future be taken into account by those who presume to be able to calculate risk to humans from animal carcinogenicity test data.

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