Laboratory approaches to the assessment of possible occupational cancer hazard

Author: Dr. Francis J.C. Roe, DM, DSc, FRC Path.
Date: 19th June, 1981


Introduction

Philosophy is held by some to be a suitable pursuit for those who are not terribly good with their hands, or for aging citizens struggling to explain away the disappointments and failures of their lives. I hold it in higher esteem, and so in approaching such a complex topic as the role of laboratory research in occupational carcinogenesis, I felt that the first step should be to map out the general position as we presently understand it. There seem to me to be 3 important basic facts. Firstly, cancer is only one of several risks to life and health associated with occupations of all kinds. Secondly, life-style factors, including dietary, smoking, sun-bathing and sexual habits, between them contribute far more than occupational factors to the overall human cancer burden. Thirdly, the number and variety of chemical and other environmental factors already known to have a real or theoretical capacity for enhancing cancer risk is such that no conceivable system of control or regulation could totally exclude them all.

In the light of these facts, the main aim of laboratory research should not be simply to pin-point more and more substances which, under one or other set of circumstances, might theoretically increase cancer risk, but to distinguish major and real cancer risk factors from minor and from purely theoretical ones. This is the kind of information needed for setting priorities by those whose job it is to try to reduce the present human cancer burden by the introduction of protective measures and regulation. Finally, I would stress one, perhaps for less obvious, philosophical principle: there is no point in time when research, either epidemiological or experimental, can be regarded as having been completed.

Periodic safety/risk assessments should, in my view, continue for as long as there are humans alive who have been exposed to an agent. Science does not stand still. New possibilities for hazard await discovery and new insights into mechanisms could show that previously perceived hazards are not real.

A great deal has been said elsewhere about the importance and need for weighing risk against benefit and I do not propose to go over this same ground
here. Obviously there can be no justification for risking anyone's health if there is no compensating benefit but it is difficult to weigh benefit to the community or to a section of the community against risk to a small number of individuals.

Concept of Carcinogenesis

That both the human body and cancers consist of cells is agreed by all. What is not agreed is whether the crucial determinant of cancer development is an event taking place in a single, initially normal, body cell. Most cancerologists conceive carcinogenesis as consisting of two processes, the first - called tumour-initiation - consisting of a mutation in a single cell, and the second - called promotion - involving a variety of ill-defined mechanisms. However, opinion is sharply divided as to which of the two processes is the more important.

This two-process concept of carcinogenesis is useful but misleadingly simplistic, particularly as far as tumour promotion is concerned. From human and experimental data, mathematicians have deduced that carcinogenesis is usually a multi-stage process and that the number of stages is usually within the range 3 - 6. Also it is clear that different types of cancer have quite different patterns of causation and that factors which contribute positively to the causation of one type of cancer may have no effect, or even the opposite effect, in relation to other types of cancer.

The situation is further confused by the fact that genetic differences are associated with differences in susceptibility, both to the 'spontaneous' development of particular cancers and to their occurrence in animals or humans exposed to known carcinogens. Some inbred strains of mice carry tumour viruses in high titre. Animals of such strains tend to develop cancers of certain kinds irrespective of environmental conditions. However, seemingly slight and non-specific changes in the environment may greatly magnify the risk of their early development of cancers.

I do not wish to add to the general confusion by introducing new concepts or schemata intended to cover all possible mechanisms of carcinogenesis. However I must just provide a skeleton for the rest of my talk. I do this in Figure 1, using the term 'genotoxic' to describe factors which cause mutations and 'epigenetic' to describe factors which enhance cancer risk by other mechanisms. The latter term applies to a wide variety of possible mechanisms, which may act
not only after but also at the same time as or even before genotoxic agents to enhance cancer risk.

An employer engaging a new member of staff inevitably takes on someone who has an average of 1 in 4 chance of developing cancer before he/she dies. Bad genes, previous or subsequent heavy smoking, exposure to industrial carcinogens, to the sun, or to various dietary factors, etc. might increase the chances above 1 in 4. Most or all of those who are not destined to develop cancers before they die from other causes will take to their graves numerous body cells that have suffered mutations and numerous precancerous lesions that have failed to develop into cancers. If these persons lived longer more of them would develop true cancers. Against this uncertain and unsatisfactory background the employer's duty is to see that he does not accelerate the rate at which his workforce suffer cellular mutations and does not increase the chances that mutant cells, or precancerous lesions, will blossom into full-bloodied cancers either while they are working for him or subsequently.

Laboratory approaches to the identification of possible carcinogenic hazards

There is all the difference in the world between testing an agent for possible carcinogenicity and assessing whether humans are or might be at increased cancer risk as a consequence of their work situation. Individually, all the tests and techniques I shall mention have their limitations when it comes to their ability to predict hazard or safety for man. The assessment of safety or hazard should take into account all the available information and not just the results of a single test or one kind of test. Moreover, evaluations should be regarded as revisable in the light of new information.

In Table 1 are listed the approaches available for detecting genotoxicity and epigenetic activity. Dr. Phillips will be talking about short-term tests for carcinogenicity and Professor Harrington will consider epidemiological approaches. After briefly discussing the value of prediction from structure and known biological activity, I propose to devote the rest of my talk to an interpretation of tests for carcinogenicity in whole animals.
What can be deduced from chemical and physical structure and from known biological activity?

Until a few years ago it was generally held that the correlation between chemical structure and carcinogenic activity is so weak that there is little to be gained by trying to predict the latter on the basis of the former. Recently, however, the position has changed dramatically with regard to the prediction of genotoxic tumour-initiating activity. The realisation that most agents which seem to enhance cancer risk only do so after they have been converted within the body to metabolites capable of reacting with DNA and a greatly expanded knowledge of metabolic activation pathways have greatly increase the accuracy of prediction of genotoxic activity from chemical structure. Indeed in the hands of specialists in this area such as John Ashby and his colleagues (1978) the accuracy of such prediction is at least as good as that provided by tests for bacterial mutagenicity.

When it comes to epigenetic mechanisms, however, prediction from chemical structure is still unreliable although it is always sensible as a first step to consider analogies - physical, chemical or biological - between a new agent and other agents known to be hazardous or safe. For example, we know that inhaled asbestos fibres enhance the risk of cancers of the lung and mesothelium by an ill-defined epigenetic mechanism. There is persuasive evidence that the long thin shape, rigid structure and insolubility of the fibres which get trapped deep in the lung are fundamental to the carcinogenic activity of asbestos. We are thereby alerted to the possibility of similar risk from the inhalation of fibres with similar physical characteristics irrespective of their chemical composition.

Tests for carcinogenicity using whole animals

There is presently a surfeit of guidelines for testing chemicals for carcinogenicity and there is no point in my going over this ground again here. Instead I propose to highlight certain important features and difficulties in interpretation, some of which are poorly addressed in many of the official publications.

Laboratory animals are not clean test tubes. Even under conditions where vigorous steps are taken to protect animals from known carcinogens, they develop tumours in high incidence. Theoretically, this may be because we have not yet identified all the carcinogens from which laboratory animals need to be protected. However, other factors seem much more important. During recent years epidemiologists, such as John Higginson of the International Agency for Research on Cancer
in Lyon, have been emphasising the relative importance of so-called 'life-style' factors in relation to cancers in humans (Higginson and Muir, 1979). The position seems to be very similar in the case of laboratory animals.

In theory, one designs a carcinogenicity test in animals such that all the animals are alike genetically and environmentally in every way except exposure to the test agent. In practice this may be difficult or impossible to do. For instance, the addition of a test material to the feed may provoke inappetance which may indirectly affect the outcome of a study. In general, the more stringent the test, (e.g. the higher the dose level or the greater the disturbance of its daily life), the greater the risk of introduction of non-specific influences, in either direction, on tumour risk. Another problem is that it simply may not be possible to expose animals to a chemical in a manner which mimics human exposure.

When, some years ago, (Davis et al (1975)) tried to produce lung tumours in female rats by exposing them to cigarette smoke by inhalation, the first problem was that there was no way of persuading the animals that the correct way to smoke a cigarette is to take puffs through the mouth at intervals. But when they were exposed via the nose most of the interesting particulate matter was arrested in the well-developed nasal turbinate system before it reached the lungs. The main finding relevant to carcinogenesis in the test was a significant reduction in incidences of mammary tumours in the smoke exposed group compared to the controls (Table 2). The authors suggested that the stress associated with exposure to irritants in smoke non-specifically protected the animals from mammary tumour development. Had the result been round the other way, anti-smoking campaigners might well have claimed that smoking produces breast cancer.

Perhaps the most remarkable thing about the Davis et al (1975) experiment was not the fact that exposure to smoke reduced the incidence of mammary tumours, but the fact that the incidence of mammary tumours was so high in the untreated control rats.

The introduction of pathogen-free animal facilities, has made it possible to keep animals into old-age without excessive losses from intercurrent infections. Partly for this reason, but mainly because of a tradition for overfeeding animals and because of the artificiality of the conditions under which we keep animals, we are currently witnessing an epidemic of neoplasia in laboratory rats and mice (Roe, 1981).
At random I plucked from the literature data for control rats in a major carcinogenicity study on the pesticide, 2,4,5-T, which I knew to have been carried out with great thoroughness (Kociba et al, 1979). High incidences of tumours of various sites was a feature of this study, and particularly noticeable was the fact that many of the tumours were of endocrine glands or of tissues under the control of sex hormones (Table 3).

I ask myself, and I ask you, can it be right to attempt to test substances for carcinogenicity in animals exhibiting a 63% life-time incidence of pituitary tumours, a 76% incidence of mammary fibroadenomas, a 51% incidence of adrenal medullary tumours, or a nearly 50% incidence of one or other type of pancreatic neoplasm? I hasten to point out that I am not specifically criticising this study on 2,4,5-T which was of exceptionally high standard. I am pressing for a radical change in the design of carcinogenicity tests based on research yet to be done.

Since 1973, I have been increasingly intrigued by the fact that it is possible to dramatically reduce the incidence of benign and malignant tumours at many sites by the stratagem of simple dietary restriction. Three tables prepared from recent publications by Conybeare (1980) and Tucker (1979) illustrate this point (Tables 4-6). For many reasons that I have no time to deal with here, I strongly suspect that the main effect of diet-restriction is not attributable to reduced intake of calories. Instead I suspect that it relates to a change in hormonal status engendered by the food-restricted animal being faced for a period during each day by an empty food hopper. An animal reacts to the absence of food by wondering what its next move should be. It becomes anxious and starts foraging as it would in the wild.

When one thinks about it, the life of animals in a typical carcinogenicity study is highly abnormal. Firstly, they are provided continuously with a surfeit of highly nutritious food – probably far too nutritious, especially if mature animals are given diets formulated for young growing animals. Secondly, they have celibacy forced on them under conditions where they can smell animals of the opposite sex but not achieve sexual fulfilment. Thirdly, they are severely deprived of exercise. Fourthly, they are deprived of certain anxieties: there are no predators to be wary of and no worries regarding food or shelter. In the bad old days some of these needs were perhaps counteracted by the host of diseases that animal stocks carried – but this is no longer the case. And so the typical aged rat or mouse these days is obese, sluggish and prey to diseases secondary to abnormal hormonal status. My plea is for
someone to undertake the research needed for defining conditions under which
laboratory animals can be maintained into old age in normal hormonal status and
without obesity.

Ad libitum feeding is unscientific and wasteful. Many of our troubles would
disappear if slightly restricted feeding were the normal practice in rat and
mouse studies. Curiously, it is already normal practice to ration the food
given to other species (e.g. dogs). Why not laboratory rodents also?

Part of the additional research that is needed relates to the composition
of diet. During recent years much attention has rightly been paid to the
exclusion of known carcinogens such as benz(a)pyrene, dimethylnitrosamine and
aflatoxin from animal diets. However, far less attention has been paid to the
suitability of diets for mature animals and the effect of dietary composition
on incidence of geriatric disease. I never cease to be astounded by data
reported by Gellatly (1975). He found that simply by doubling the concentration
of ground nut oil (GNO) in a carcinogen-free semi-synthetic (S.S.) diet he could
multiply the risk of liver tumour development by a factor of over 5 (see Table7)

At present when the epidemiologists talk about the importance of life style
factors in the causation of human cancer, one is apt to be irritated by their
vagueness. And yet, until further properly designed research is undertaken, the
nature and importance of life-style factors in the causation of tumours in
untreated control rats and mice is an equally vague topic.

Interpretation of carcinogenicity tests in animals

I hope I have said enough to impress those unfamiliar with the subject that
the design, conduct and interpretation of carcinogenicity studies in animals is
not necessarily simple and straightforward. However, there is one more
important point that needs to be stressed. A positive result in an animal
carcinogenicity test does not enable one to distinguish between an initiating
genotoxic carcinogen and an agent that enhances tumour risk by an epigenetic
mechanism. Since control animals carry tumour viruses and are inevitably (despite
all the efforts made to prevent it) exposed to background carcinogens such as
cosmic rays, and since, under present conditions at least, they are destined
to develop all sorts of tumours as a consequence of overfeeding and disturbed
hormonal status, it is simply not justifiable to conclude that an increased
incidence of tumours in a treated group is attributable to the initiation of the
extra tumours via a genotoxic mechanism by the test agent. But let me be more helpful - If the effect of a test agent is simply to enhance the risk of development of a tumour which is occurring frequently in untreated control animals, then the mechanism is quite likely to be epigenetic. However, if treated animals develop tumours of a kind that are very rare in control animals then a genotoxic mechanism is more likely.

As I said earlier, the evaluation of a substance for carcinogenicity should be based not on the results of isolated tests but on a consideration of all the available information. If, for instance, we have evidence from in vitro tests that a chemical has genotoxic potential, this makes it more likely that positive results in an animal study are indicative of true carcinogenicity. On the other hand, a substance such as saccharin which is neither genotoxic nor converted to a genotoxic metabolite, but which, in high dose, increases bladder tumour risk in rats, is most probably producing its latter effect by an epigenetic mechanism.

Statistical considerations

It is all too easy to overlook the fact that the most reliable epidemiological cancer data relate to mortality. Whereas most of the tumours reported in many carcinogenicity studies relate to small tumours discovered incidentally in animals dying from other causes. These types of data are very different. Recently, under the driving force of Richard Peto, the IARC (1980) published guidelines for the handling of tumour data from animal carcinogenicity studies. The most important feature of these guidelines is that they outline different methods for dealing with data for fatal tumours and for tumours discovered incidentally in animals dying from other causes. Totally misleading conclusions can be made if this distinction is not made in the analysis of data and if tumour incidences are not age-standardized. One slight problem in this connection has been that some experimentalists feel that it is rarely possible to determine accurately the cause of death of a laboratory animal and therefore it is not possible to distinguish incidental from non-incidental neoplasms. While I have sympathy for this view I do not think it detracts from the importance of attempting to make the distinction as best one can and the Guidelines suggest how one may go about doing this.

Conclusion

Although I have spent more time discussing the difficulties of designing animal tests and their interpretation than their merits, I continue to believe that they have
an essential place in the evaluation of chemicals and other environmental agents for possible human cancer hazard. They should not be regarded as less fallible than other kinds of test. They overcome many of the important drawbacks of in vitro test systems, but introduce some of which in vitro systems are free. Overall there is a comity between animal species such that they tend to react in similar ways to foreign stimuli. This comity is seemingly greater for genotoxic than epigenetically acting agents. An adverse finding in an animal carcinogenicity study should usually be regarded as predictive of what would happen in man under similar conditions of exposure unless and until there are good grounds for concluding otherwise.
References


Figure 1: Mechanisms in carcinogenesis

1 or more mutations lead to a cancer cell with one or more mutations. This mutated cell can be influenced by genotoxic agents or epigenetic factors. No problem occurs if the cell is killed or persists without problems. (Influenced by epigenetic factors)
Methods for identifying factors which enhance cancer risk:

I  **Genotoxic mechanism**

Prediction from structure and known biological activity

Tests for genotoxicity

(a) at gene level (base-pair change and frame-shift mutations in bacteria or mammalian cells)

(b) at chromosome level (mammalian cells, animals, humans)

Life-span studies in animals*

Clinical and epidemiological studies*

* Do not distinguish between genetic and epigenetic mechanisms.

II  **Epigenetic mechanisms**

Prediction from structure and known biological activity.

In vitro methods

Special tests in animals (e.g. Tests for enzyme induction, tumour promotion, cocarcinogenicity, immunosuppressive activity, hormonal activity)

Life-span studies in animals*

Clinical and epidemiological studies*

* Do not distinguish between genetic and epigenetic mechanisms.
Table 2

Effects of Life-time exposure of female Wistar rats to cigarette smoke

(from Davis et al, 1975)

<table>
<thead>
<tr>
<th></th>
<th>Squamous</th>
<th>Mammary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of</td>
<td>tumours</td>
</tr>
<tr>
<td></td>
<td>rats</td>
<td>of lung</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoke exposed</td>
<td>408</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham exposed</td>
<td>102</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>N.S.</td>
<td></td>
</tr>
</tbody>
</table>

* Observed
* Expected based on age-standardized incidence in the whole study which included 3 other groups.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Male (%)</th>
<th>Female (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pituitary</td>
<td>31</td>
<td>63</td>
</tr>
<tr>
<td>Adrenal - cortex</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Adrenal - medulla</td>
<td>51</td>
<td>8</td>
</tr>
<tr>
<td>Thyroid - C-cell</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Parathyroid</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas - exocrine</td>
<td>33</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas - endocrine</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>Testis</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Ovary</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>Mammary gland - fibroadenoma</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Mammary gland - adenoma</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>Mammary gland - other</td>
<td></td>
<td>29</td>
</tr>
</tbody>
</table>

(from Kociba et al, 1979)
Table 4. **Effect of simple dietary restriction on tumour incidence in mice**

(Values are no. of mice which developed tumours at any time during the study. There were 160 mice of each sex in each group.)

<table>
<thead>
<tr>
<th>Feeding regimen ...</th>
<th>Type of tumour</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ad lib.</td>
<td>Restricted to 75% of ad lib.</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>30</td>
<td>19*</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>47</td>
<td>12***</td>
</tr>
<tr>
<td>Lymphoma</td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Any tumour at any site</td>
<td></td>
<td>71</td>
<td>36***</td>
</tr>
<tr>
<td>Any malignant tumour</td>
<td></td>
<td>17</td>
<td>7*</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001.
†Conybeare, 1980.

Table 5. **Effect of dietary restriction on 'spontaneous' tumour incidence in rats**

<table>
<thead>
<tr>
<th>Feeding regimen ...</th>
<th>Food consumption (g/d)</th>
<th>Survival for 2 years (%)</th>
<th>Tumour-bearing animals before or at 2 years (%)</th>
<th>Mean number of tumours/rat</th>
<th>Males</th>
<th>Females‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ad lib.</td>
<td>Restricted</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>72</td>
<td>66</td>
<td>0.94</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>90</td>
<td>24</td>
<td>0.27</td>
<td>82</td>
<td>56*</td>
</tr>
</tbody>
</table>

*P<0.05, **P<0.01, ***P<0.001.
†Tucker, 1979.
‡The ad lib. fed females ate less than anticipated and in fact consumed only the same amount daily as the restricted animals. The difference was that the ad lib. fed animals were never faced with an empty food basket.

Table 6. **Effect of dietary restriction on incidence of pituitary and mammary tumours in rats**

<table>
<thead>
<tr>
<th>Feeding regimen ...</th>
<th>Rats with pituitary tumours (%)</th>
<th>Rats with mammary tumours (%)</th>
<th>Males</th>
<th>Females‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad lib.</td>
<td>Restricted</td>
<td>Ad lib.</td>
<td>Restricted</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>0***</td>
<td>66</td>
<td>39**</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
<td>34</td>
<td>6***</td>
</tr>
</tbody>
</table>

**P<0.01, ****P<0.001.
†Mary Tucker, unpublished results.
Table 7  Dietary fat and liver tumours in C57BL female mice

<table>
<thead>
<tr>
<th></th>
<th>Benign or malignant</th>
<th>Malignant</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS diet with 5% GNO</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>SS diet with 10% GNO</td>
<td>43</td>
<td>9</td>
</tr>
</tbody>
</table>

GNO, groundnut oil.
*Gellatly, 1975.