CHAPTER 1

GENERAL INTRODUCTION AND OUTLINE

Animal experimentation in cancer research dates back to 1775, when Peyrithe injected human cancerous material into a dog in an attempt to induce neoplasms (Shimkin, 1977). Since then, many attempts have been made to transplant human tumours to animals, and the first successful experiment of this type was reported by Nowinsky (1876). The first use of animals in the field of chemical carcinogenesis is attributed to Yamagiwa and Ichikawa (1915). By the application of crude coal-tar dissolved in benzene to the ears of 137 rabbits every two or three days, they induced 'folliculo epitheliomas' in nearly all ears after a period longer than 100 days, and, within a year, seven cases of fully developed invasive carcinomas occurred. The relevance of animal carcinogenesis experiments to man was demonstrated by the induction of bladder tumours in dogs exposed to aromatic amines (Hueper & Wolfe, 1937; Hueper *et al.*, 1938); these results corresponded to epidemiological observations that workers exposed to similar agents in the workplace had an increased occurrence of bladder neoplasms.

Since then, long-term animal experiments have become an integral part of cancer research. They are used for various purposes, including screening chemicals or other exposures (for example, ionizing radiation) for their carcinogenic potential and for elucidating the mechanisms of carcinogenic action of known cancer-causing agents. Their importance in the process of assessing cancer risks has been discussed repeatedly (see, for example, Tomatis, 1977, 1979; Weinstein, 1981), and even with the increasing use of short-term mutagenicity assays, animal experiments remain an indispensable component of cancer risk assessment.

A review of the *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans* indicates that in the first 32 volumes published between 1971 and 1983, 654 chemicals, groups of chemicals, industrial processes or occupational exposures were considered. Epidemiological data were available for only 157 of these; an assessment of these data led to the classification as carcinogenic to humans of seven industrial processes or occupational exposures and 23 chemicals or groups of chemicals. However, considerably more data on the cancer risk of these 654 exposures were available from long-term animal experiments. Based on animal data, there was sufficient evidence of carcinogenicity for 187 chemicals, limited evidence of carcinogenicity for 176 chemicals, no evidence of carcinogenicity for eight chemicals and the data were inadequate to evaluate 260 chemicals. Most known human carcinogens have been found to be carcinogenic in experimental animals by appropriate testing,

and in many cases the experimental evidence preceded the epidemiologic evidence of human risk.

The use of long-term animal experiments for routine testing of chemicals has become part of the procedure for setting public health regulations in many countries. The conduct of carcinogenicity studies is often required for the registration of new compounds, and compounds already in use are being progressively considered for screening. For example, in the USA the Bioassay Program of the National Cancer Institute investigated several hundreds of compounds, and further studies are now in progress under the National Toxicology Program with about 25 long-term carcinogenicity studies started every year. It is difficult to obtain an exact number of such studies undertaken worldwide each year, but an estimate of the order of magnitude of several hundred may be appropriate. In the *Information Bulletin on the Survey of Chemicals Being Tested for Carcinogenicity* (IARC, 1982a), about 1000 chemicals were reported to be under test in 103 institutes in 16 countries.

A number of methodological improvements have led to the establishment of long-term animal experiments as an indispensable part of cancer research. Four areas of improvement, in particular, may be identified. Many authors view the control of genetic variability by the introduction of inbred strains as a progressive step in biomedical research (for an interesting review, see Festing, 1979). The control of genetic variability allows for the reproducibility of the experiments, an important criterion in experimental science.

Advances have also been made in the design or conduct of the experiments. Minimal requirements for an acceptable protocol have been established for several aspects of carcinogen bioassay experiments; these include the number and size of the experimental groups, the selection of dose and route of administration, randomization, animal husbandry, diet, duration of exposure and observation period (Interagency Regulatory Liaison Group, 1979; IARC, 1980). Currently, the role of diet in long-term animal experiments is a key topic for discussion.

Thirdly, the pathological evaluation of experimental animals has undergone considerable change. Careful macroscopic inspections, standardized histology and broad pathological reviews have contributed to improvements in the quality of pathological data (Interagency Regulatory Liaison Group, 1979). The experimental pathologist's work may be organized very differently in different settings, depending on the size of the laboratory, the design of the experiments and related aspects, which will be discussed in Chapter 3. Whereas some pathologists observe the animals from the beginning of the experiment to the final evaluation, thus being familiar with all clinical signs, others conduct the final review without such information. The question as to whether pathological reviews should be conducted blindly, that is, without knowledge of an animal's treatment group, has been a very controversial subject which remains not fully resolved (Weinberger, 1973; Fears & Schneiderman, 1974; Ward *et al.*, 1978).

Finally, statistical methods used to analyse such experiments have improved considerably. With the realization that differences in intercurrent mortality have to be adjusted for in the comparison of proportions of tumour-bearing animals, and the development of specific models to describe various aspects of the carcinogenic process, the range of statistical methods available has expanded greatly.

The purpose of this book is to provide a comprehensive introduction into all statistical aspects of long-term animal experiments. A variety of methods published recently in the scientific literature are summarized comprehensively. This is intended mainly to help statisticians working in the field of long-term animal experiments, as well as helping experimentalists with an interest in the quantitative interpretation of their results to familiarize themselves with the methods available. However, as the first three chapters provide a nontechnical introduction to major concepts related to the statistical evaluation of long-term animal studies, the book will be of interest to a much wider audience of scientists, public health officers and others who are concerned with long-term animal studies. Finally, it can also serve as a text for courses taught to students in biostatistics or a related medical field.

Throughout the book, emphasis is placed on the occurrence of tumours as the basic endpoint in long-term animal experiments. However, some attention is also given to the many other observations that have to be taken into consideration for the statistical assessment of a carcinogenic effect.

With these aims, this book complements Supplement 2 to the *IARC Monographs* series, entitled 'Long-term and short term screening assays for carcinogens: A critical appraisal'. That publication includes a section entitled 'Basic requirements for long-term assays for carcinogenicity' and an Annex, 'Guidelines for simple, sensitive significance tests for carcinogenic effects in long-term animal experiments'. Whereas the section on basic requirements mainly addresses the conduct of the experiments, the annex on statistical guidelines describes in non-technical terms one method for routine analyses of carcinogenicity experiments. This method is also described in this monograph, where it is included in a general and more technical description of the methods available. In addition, the current monograph discusses the circumstances under which the various methods are suitable and the assumptions that underlie them.

The contents of the remaining chapters are as follows. In the second chapter, general considerations related to the statistical evaluation of long-term animal experiments are discussed. The third chapter deals with various aspects of the experimental design. In the fourth chapter, some illustrative data sets are introduced which serve throughout the monograph as examples. The fifth chapter then discusses methods available for comparing tumour occurrence in different experimental groups. The sixth chapter outlines the fitting of statistical models, which are useful in summarizing patterns of tumour incidence and in attempting to elucidate mechanisms of action. The seventh chapter is devoted to a list of special topics, all of which are important in this field and deserve careful attention; they include multiple statistical comparisons, multiplicity of tumours, litter effects, association among tumour types, the use of historical controls and multigenerational studies. Finally, in the eighth chapter, we deal with the analysis of concomitant observations such as survival rates, body weight and food consumption, clinical signs of toxicity, haematological variables, and organ weight.