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PART III. MECHANISMS OF CARCINOGENESIS
METHODOLOGY OF EXPERIMENTAL CHEMICAL CARCINOGENESIS

Experimental Assessment of Carcinogenicity

In order to determine the possible carcinogenicity of tobacco smoke constituents, the same procedures should be employed as are used for other substances. Various criteria and guidelines for carcinogenicity tests have been advocated by several governmental and international agencies and by various advisory groups. For example, the World Health Organization (WHO) (29), the International Agency for Research on Cancer (IARC) (8), the Environmental Protection Agency (3), the Food and Drug Administration (4), the National Cancer Institute - National Toxicology Program (22), Health and Welfare of Canada (2), and the Health Council of the Netherlands (13), as well as others, have issued guidelines for the testing of compounds for different aspects of acute and chronic toxicity.

Chemicals

As a first step in the testing of any material for possible carcinogenicity, the researcher should obtain a complete physico-chemical characterization of the material. Examinations by such techniques as thin-layer, gas-liquid, or high performance liquid chromatography should afford some idea of whether the material is homogeneous or a mixture of components. If the last is the case, identification of the individual components and determination of the level of each in the mixture are highly desirable. Otherwise, the validity and significance of the results may be questioned.

Factors Influencing Carcinogenicity

In tests for possible carcinogenicity, several factors influence the outcome of any study. Those relevant to the compound are the route of administration and the dose and frequency of administration. Factors relating to the animal are the species, strain, sex, age, diet, spontaneous tumor incidence, and immunological status.

Route of Administration

Oral administration

In addition to being a logical technique for testing compounds that may be ingested by humans, oral administration is also useful for compounds that may be inhaled as dusts, cleared from the airways by ciliary action, and then swallowed. Compounds may be mixed in the feed, given as aqueous solutions instead of normal drinking water, given by gavage at appropriate intervals, or even given in capsules. If the compound is mixed with the feed, the uniformity of
mixing, the stability in the diet, and the nonreactivity with the feed are factors of concern. Volatile compounds should not be given in the diet, for the resultant loss will lead to inaccuracies in dose levels. If given in the drinking water, solubility and stability must be considered.

**Dermal**

The dermal route simulates exposure of the skin as it occurs in occupational situations or in the use of cosmetics, and has been used as a standardized carcinogenicity assay. Application of a solution of the test material by means of a pipet should be made in an area that cannot be reached by the animal. Otherwise, the animal will lick the treated area so that oral ingestion occurs. To avoid the animals' licking each other, single caging is desirable. In this type of test, mice, hamsters, rabbits, and sometimes rats are used. For cutaneous application, mice of the BALB/c, C3Hf, or DBA strains or the non-inbred Swiss strain are most responsive. SENCAR mice have been especially bred for sensitivity in initiation-promotion assays. The skin should be clipped before application of the test compound, but abrasion or mechanical injury of the skin should be avoided.

**Implantation: Subcutaneous and Intramuscular**

Although subcutaneous injection of polycyclic aromatic hydrocarbons in mice has proved to be quite reliable as a test system, the use of this test in other species has led to controversial results. The induction of tumors at the implantation site, especially in rats, by inert materials of the proper size, by saline solutions, or by oily solvents has indicated the limitation of this test.

**Injection: Intraperitoneal and Intravenous**

Intraperitoneal and intravenous injections may be used to test drugs, but for various reasons are not suitable for repeated dosing. They are useful for administering a single dose or a few doses of potent carcinogens for model experiments. With this technique, exposure of personnel to carcinogens is minimized.

**Inhalation**

Inhalation is the major route by which persons are exposed to cigarette smoke. For laboratory study, complex installations, such as pumps or metering devices, are needed to allow uniform delivery of the test material to the experimental animals. Scrubbers and other devices are required to prevent exposure of any personnel working in the area. A test by the inhalation route usually costs much more than studies using other routes of administration.
In lieu of using large inhalation chambers in which animals are exposed, it is possible to use chambers into which the head and nose of individual animals are fitted. The test material is then forced into the chamber, resulting in an inhalation exposure. Relatively few animals can be treated with a given chamber by this method, however.

Factors that should be considered in evaluating the results of the test are effects on secretion of mucous, alteration of pulmonary ventilation, and possible toxicity to the cilia in the respiratory tract.

The dilemma is that in rodents the anatomy and physiology of the respiratory tract and the biochemistry of the lung differ from that of humans and that animals anatomically resembling the human most closely are too expensive and have lifespans too long to permit their use in routine tests.

For inhalation tests of the carcinogenicity of tobacco smoke and various fractions of tobacco smoke, hamsters are preferable to rats and mice because they respond with a higher incidence of airway tumors (6).

Higher dose levels, greater frequency of administration, and longer periods of observation are required for weak carcinogens than are needed for potent ones. For example, potent carcinogens such as 7,12-dimethylbenz[a]anthracene or nitrosomethylurea can induce cancers in certain animals after a single dose. On the other hand, a single or very low dose of compounds such as N-2-fluorenylacetamide, safrole, and dioxane may not lead to tumors within the lifespan of the animal.

**Animal Factors**

**Species**

The choice of species rests on several factors, including lifespan, size, sensitivity to a specific class of compound, and availability. Early studies on skin painting of benzo[a]pyrene showed that mice and rabbits were responsive, while the few other species tested were less responsive. Guinea pigs are not suitable for testing aromatic amides and amines or their precursors. They either lack the enzyme system that activates aromatic amines or degrade the activated metabolite so rapidly that there is no effect. Overall, mice are the most useful animals for skin painting bioassays; rats are useful for test material that might be fed, especially with nitroso compounds or aromatic amines; and hamsters seem better suited for inhalation studies on tobacco smoke or its components.

Larger species including the rabbit, dog, and primate require a longer time to obtain results; they are expensive to purchase, to maintain, and to test; and they are not always readily available.