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BIOASSAY OF 5-AZACYTIDINE FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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BIOASSAY OF

5-AZACYTIDINE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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II. MATERIALS AND METHODS

A. Chemical

5-Azacytidine, which is a common name for 4-amino-1-beta-D-ribofuranosyl-1,3,5-triazine-2(1H)-one, was obtained as a single batch (Lot No. AP-V-128) from Ash-Stevens, Inc., Detroit, Michigan, by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (NCI).

The identity and purity of the chemical were confirmed in analyses at Stanford Research Institute. Elemental analyses (C, H, N, O) were correct for $C_8H_{12}N_4O_5$, the molecular formula of 5-azacytidine. Infrared, ultraviolet, and nuclear magnetic resonance spectra were as expected for this chemical and were identical to spectra of a reference standard. No free 5-azacytosine or D-ribose was detected by paper chromatography. On the basis of these results, the purity was estimated to be > 99%.

The powdered 5-azacytidine was stored at 5°C in small bottles enclosed in sealed plastic bags containing Drierite®.

B. Dosage Preparation

Concentrations of 5-azacytidine of 0.1 or 0.2% for rats and 0.02 or 0.04% for mice were prepared in buffered saline (pH 6.9) for intraperitoneal injection of the chemical. Aqueous solutions

were not stored, because they are unstable at room temperature. The drug and the vehicle were mixed in a 10-ml glass Potter-Elvehjem tissue grinder with a Teflon pestle. Fresh solutions in exact amounts for each administration were prepared preceding injection.

C. Animals

Female Sprague-Dawley rats and male and female Swiss mice were used in subchronic studies.

Sprague-Dawley rats and B6C3F1 mice of both sexes, obtained through contracts of the Division of Cancer Treatment, NCI, were used in chronic studies. The Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, and the B6C3F1 mice were obtained from Charles River Laboratories and from A. R. Schmidt, Madison, Wisconsin. On arrival at the laboratory, the male rats were 30 days old, the female rats were 37 days old, and the mice were all 30 days old. The animals were quarantined for an acclimation period (rats for 5 days, mice for 4-5 days), assigned to control and treated groups, and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled