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PROTOCOL FOR
A CARCINOGENICITY STUDY
USING NEONATAL MICE

I. Introduction

The use of newborn mice in evaluating the carcinogenicity of polynuclear aromatic hydrocarbons has been reported by Roe, et al (Brit. J. Cancer 15:515:1961), Pietra and Shubik (Cancer 14:308:1961), Kelly and O'Gara (J. Nat. Canc. Inst. 26:651:1961) and others. Dimethylbenzanthracene, benzpyrene and methylcholanthrene were used to obtain lymphomas and pulmonary tumors. Correlations between age of the animal at time of treatment and the length of the carcinogenic induction period along with quantitative and qualitative measurements of tumorous tissues produced have been obtained. Dose response studies have indicated that larger, sublethal doses are able to decrease the induction period and to increase the incidence of cancerous lesions with regard to both number of animals affected and number of lesions per animal. However, at this time there has been no definite experiment to show that, at some lower dosage level, a carcinogenic response is absent.

There is cause to suppose that this technique would be equally valid for a study of non-hydrocarbon compounds.

II. Experimental

Swiss white mice will be employed as the test species; 25 pregnant animals will be obtained and allowed to deliver normally. The newborn animals, less than 24 hours of age, will be those used in the actual experiment. A total of 160 newborn, selected randomly from the 25 litters, will be employed in the study, divided into two groups, one experimental group and one treated control group. Each group will contain 80 animals, 40 of each sex. Since a number of newborn will not survive to weaning in the normal condition and since handling of the young is known to exert some selective pressure against survival, it is expected that at weaning (21 days post-partum) each group will contain 60 animals (75% survival). If test material toxicity is observed and survival is lowered, additional animals will be treated in an attempt to obtain at least 60 animals per experimental group.

The test compounds will be administered to animals less than 24 hours old by direct intraperitoneal injection. A 27-gauge needle attached to a one-milliliter syringe will be employed, with all materials to be injected contained in 0.2 milliliters of a selected solvent. To restrict cage losses and maternal cannibalism, gloves will be worn at all times during the treatment period. Newborn will be returned to their original litters following treatment and will remain until separated into the various experimental groups at weaning.

The materials to be tested will be:

1. Triclocarban Solution
2. Solvent

All animals will receive one dose at birth followed by daily observation for survival, general appearance and external signs of neoplastic growth. At 26 weeks, one-half of the animals in each group will be sacrificed and examined for evidence of a tumor growth. The remaining animals will be sacrificed at 52 weeks (unless it is felt advisable due to good survival, lack of apparent tumorgenicity that the study be extended to 78 weeks).

Special attention will be paid to the evaluation of pulmonary tissues at sacrifice. The lungs will be fixed while fully expanded by anesthetizing the animal (Brevital Sodium), and placing a loose loop around the trachea. The animal will then be exsanguinated by cutting the aorta through an opening in the abdominal wall and drawing the tracheal ligature tight. The lungs will be carefully excised and immersed in 10% buffered formalin. Paraffin sections from all lobes, as well as the trachea, and regional lymph nodes will be obtained and pathologic examination conducted.

A complete histologic evaluation will be conducted on every animal following sacrifice or death. The examination will include description and classification of tumorous tissue.

At the conclusion of the experiment, a complete formal report will be prepared which will include statistical evaluation relative to carcinogenic induction times, the incidence of lesions, the severity of lesions and the compatibility of the observed lesions with survival.

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