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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.

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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.

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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.

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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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PROTOCOL FOR

TERATOGENIC SCREENING STUDY

ALBINO RATS AND SWISS WHITE MICE

The teratogenic potential of a chemical may be detected in model animal systems. Variable responses to teratogenic agents are obtained when different species, dosing schedules and dosage levels are employed. In order to minimize the contribution of these experimental variables and to ascertain whether or not a chemical does possess the ability to induce congenital malformations in mammals, the following protocol is suggested. Two random-bred strains of rodent, the albino rat and the Swiss white mouse, whose genetic backgrounds are such that reproductive parameters, egg released per mating, percentage of fertilized eggs and number of offspring per litter are well-established, will be selected for the experiment. All animals will be mated and females showing positive signs of mating following vaginal cytologic evaluation will be employed, the day of sperm-positive findings being designated as Gestation Day Zero.

The test chemical will be dissolved in sesame oil and will be administered intravenously, volume of injectable material being held constant at 0.1 milliliter, as a thirty percent emulsion of sesame oil in physiological saline. Each treatment group will consist of ten pregnant animals.

Tolerable doses of the chemical will be determined after obtaining intravenous LD_{50} values for both mice and rats. The greatest dose at

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which no mortality is obtained will be employed in the first phase of the experiment. (This value will not necessarily be the same for rats and mice.) Single, intravenous injections will be given to separate groups of ten mice and ten rats at each day of the period of organogenesis (Day 3 to 13 in the mouse, Day 4 to 14 in the rat), and the animals will be allowed food and water ad libitum. Control animals will receive equi-volume injections of the solvent on the same dosing schedule so that a total of 220 pregnant females will be employed for each rodent species. All animals will be sacrificed prior to natural delivery, mice on Gestation Day 18, rats on Gestation Day 20, and the number of implantation sites, resorption sites and fetuses will be recorded. Each fetus will be examined under a dissecting microscope to detect external abnormalities or to ascertain freedom from such congenital anomalies. One-half of the fetuses will be examined for skeletal defects (following clearance of tissues with potassium hydroxide and staining with Alizarin Red), and the others will be examined for soft-tissue anomalies (free-hand razor sections following fixation in Bouin's solution).

The incidence of resorption and abnormal young will be plotted against embryonic age at treatment. From these data, the embryotoxic (causing an increase in fetal resorption) response will be measured and the earliest Gestation Day at which resorption in the test animals approached that seen in the controls will be selected to obtain information relating to a teratogenic dose response.

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Four dose levels will be selected (based on data obtained in the first portion of the experiment) and groups of ten pregnant animals plus one control group will be dosed, maintained, sacrificed and examined as before. From this information, the effect of varying the dose of the chemical at an embryo-sensitive period will be ascertained. All results will be examined statistically to determine the significance of the finding(s) obtained.

The effect of repeated doses of the chemical during gestation will be measured by selecting three dose levels and one control (ten animals per groups) and treating the pregnant animals throughout the period of organogenesis (Day 3-13 in the mouse, Day 4-14 in the rat). In this phase of the study, a third species, the rabbit, will be employed, doses given from Day 6 to 16 and sacrifice conducted on Day 29. All other observations will be the same and all animals and fetuses will be examined in the manner described earlier.