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GUIDELINES FOR CARCINOGEN BIOASSAY IN SMALL RODENTS

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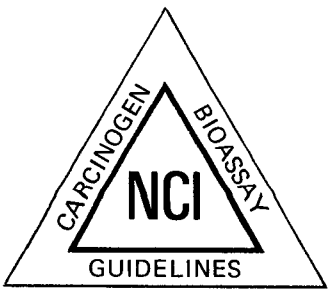
James M. Sontag, Ph. D.
Manager, Bioassay Operations Segment

Norbert P. Page, D. V. M.
Chief, Carcinogen Bioassay and Program Resources Branch

Umberto Saffiotti, M. D.
Associate Director, Carcinogenesis Program and
Chief, Experimental Pathology Branch



CARCINOGENESIS PROGRAM
DIVISION OF CANCER CAUSE & PREVENTION
NATIONAL CANCER INSTITUTE
BETHESDA, MARYLAND

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PREFACE

The National Cancer Act of 1971 provides legislative authority for the National Cancer Institute to plan and develop an expanded, intensified, and coordinated cancer research program. An urgent and essential part of this program is the need to protect the public from chemical and physical carcinogenic hazards and their effects. In an effort to meet this need, the Carcinogenesis Program of the Division of Cancer Cause and Prevention has undertaken as one of its major activities the identification of carcinogens in the environment and workplace. The responsibility to develop and maintain this activity has been assigned to the Bioassay Operations Segment in conjunction with the Carcinogen Bioassay and Program Resources Branch.

It is the purpose of this document to provide guidelines for the bioassay of chemicals for carcinogenic potential in small rodents. The initial draft of this document was written following a workshop at which the Carcinogenesis Bioassay Program's protocols were reviewed by experts in the various scientific disciplines applicable to carcinogen bioassay. The recommendations made at the workshop were reviewed by investigators active in the NCI Bioassay Collaborative Program. Later draft versions were reviewed by members of the scientific community at large. Included among the latter were workers affiliated with universities, other Federal agencies, commercial organizations, and trade associations, as well as ones from institutes and laboratories located abroad. An attempt was made to incorporate as part of this document as many of the reviewers' comments as were compatible with its intent. As a result of this extensive review by scientists representing diverse interests in our society, it is hoped that these guidelines will serve as the basis to standardize those aspects of a large-scale carcinogen bioassay which are essential to its scientific acceptability.

Appreciation is expressed to the individuals who participated in the Workshop on Carcinogen Bioassay Protocols held November, 1973, at the National Cancer Institute, Bethesda, Maryland. They were:

Richard R. Bates, National Cancer Institute
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Elizabeth K. Weisburger, National Cancer Institute

*Deceased

We also express our gratitude to the many individuals who have reviewed the material contained in this document and provided us with their comments. These comments have added greatly to the overall quality of the guidelines. In addition, we thank the many NCI staff members who have labored not only over difficult concepts but also over the sometimes more difficult task of putting them into clear and meaningful statements. In this regard, special recognition is given to Dr. Cipriano Cueto.

Questions or comments regarding the material contained in this document may be addressed to: Dr. James M. Sontag, Manager, Bioassay Operations Segment, Landow Building, Room A-306, National Cancer Institute, Bethesda, Maryland, 20014.

TABLE OF CONTENTS

I. INTRODUCTION

Most human cancers are believed to be caused by exposure to extrinsic factors, among which chemical agents are thought to be a major contributor. These agents must be identified, evaluated, and controlled if the incidence of human cancer is to be reduced. For this reason and the fact that many of these chemicals may have great social and economic impact, it is essential that the procedures used to determine their carcinogenicity be established on the best scientific bases as are practically possible. However, differences in scientific approaches and endpoints, as well as economic considerations, exclude the use of any single set of procedures to meet the objectives of all carcinogen bioassay studies. Notwithstanding these differences, certain features are common to all well designed and properly conducted long-term animal studies.

The guidelines contained herein are used by the *NCI CARCINOGENESIS BIOASSAY PROGRAM (CBP)* to screen environmental and occupational chemicals for carcinogenicity by their oral administration to small rodents. However, they also may be applicable, in part or *in toto*, to other long-term animal studies that have different procedures, objectives, or endpoints. It is not the intent of this document to address these variations. Although there are no substitutes for good animal care practices, deviations from these guidelines may sometimes be necessary for those laboratories not specially equipped to conduct large-scale bioassay studies.

II. GENERAL

The recommendations outlined in the *Guide for the Care and Use of Laboratory Animals*[1] should be adhered to throughout the bioassay study. The general areas covered are: 1. Laboratory Animal Management, 2. Laboratory Animal Quality and Health, 3. Personnel, 4. Use of Laboratory Animals, and 5. Physical Plant. When more rigid or specific standards should be applied they are noted in the appropriate sections of this document.

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1. Prepared by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council, DHEW Pub. No. (NIH) 74-23, Superintendent of Documents, U.S. G.P.O., Washington, D.C., 20402.

III. ANIMAL HUSBANDRY

III.A. *Source* Although it is ideal to have an animal production colony associated with a large-scale bioassay operation, animals supplied from commercial stocks are acceptable, providing they are high quality, disease free, genetically stable, and adequately identified as to colony source.[2] Animals should be shipped in sturdy, compartmented, and filtered containers. Arrangements should be made with the carrier to assure rapid and optimal shipping conditions that minimize trauma and extremes in temperature. Prompt notification of the animals' arrival should be given by the carrier to the receiving laboratory. Animals should be picked up immediately and placed into quarantine.

The animal strain/species used to study a test agent and its appropriate controls should be supplied from the same source. When animals from different sources are used to study different test agents, they should not be mingled and, optimally, should be maintained in separate rooms.

III.B. *Distribution* During the quarantine period and acute toxicity and repeated-dose studies, animals may be caged together according to the weight-space specifications (Appendix A) recommended in the *Guide for the Care and Use of Laboratory Animals*. However, for the subchronic and chronic studies, animals should be distributed from the outset of the studies as if they were in the upper weight range. This will obviate the need to later redistribute them to remain within the weight-space specifications. Even though the space to weight specification may be exceeded during the subchronic and chronic studies, no cage should contain more than five animals. As animals die or are sacrificed, surviving animals should not be combined or redistributed among the cages.

III.C. *Quarantine* The quarantine area should be physically separated from the testing area. Newly arrived animals should be taken, in their unopened shipping containers, directly to the quarantine area. A separate quarantine area should be provided for each species. Animals that are unsuitable by reason of size, health, or other criterion should be immediately discarded. Animals should be quarantined a minimum of seven days, after which they

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2. Most mice and rats now used come from production colonies developed and monitored by the CBP. These are periodically restarted with breeders obtained from the NIH Division of Research Services.

should be reexamined and those unsuitable should be discarded. A small, randomly selected number of animals from each shipment should be sacrificed and examined for parasites, enteric pathogens, and other diseased conditions.[3] When an epizootic disease is found among the animals, the entire shipment from which they came should be discarded. The quarantine area should be disinfected prior to the receipt of additional animals.

III.D. *Strain/Species* Both sexes of at least two species should be used for the bioassay of each test agent.[4] Considerations in selecting the proper species and strains should include the spontaneous tumor incidence, sensitivity to tumor induction, availability, genetic stability, hardiness, and longevity.

III.E. *Animal Facilities* The physical design and maintenance of the animal facilities are of great importance in assuring high standards of animal care, chemical and biological hazard control, and the proper conduct of the bioassay studies.

III.E.1. *Physical Plant* The animal facilities should be designed and operated to minimize the introduction of external biological and chemical agents into the building, as well as between individual animal rooms. The animal facilities should be effectively separated from the offices, laboratories, and other rooms that are not essential to the maintenance or treatment of the animals. The "clean-dirty" corridor flow should be used to minimize the inadvertent transfer of contaminants into the animal rooms. Service areas should be located to effectively support the animal facilities.

III.E.2. *Construction and Materials* The construction of the building and the materials used should facilitate the sanitation of the bioassay operation. Utilities such as water and electric lines and ventilator ducts should be located outside of the animal rooms. Components that interrupt the surface integrity

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3. See *Guide to Infectious Diseases of Mice and Rats*. A report of the Committee on Laboratory Diseases, Inst. Lab. Animal Resources, NRC, NAS, 2101 Constitution Ave., Washington, D. C., 20418; Pub. ISBN 0-309-01914-1.

4. The CBP uses primarily the B6C3F_1 hybrid (C57BL/6 x C3H/f) mouse and either the Fischer strain 344 or Osborne-Mendel rat.

of a room, e.g., electrical outlets and light fixtures, should be recessed into the surface and sealed. Seamless materials that are durable, waterproof, and fire-resistant should be used for interior surfaces. Paint and glazes should be highly resistant to chemical solvents, scrubbing, high-pressure sprays, and impact. Floor drains and windows are not necessary in facilities housing small rodents. Where they already exist, they should be blocked and sealed.

III.E.3. *Animal Rooms* The size and number of animal rooms should be sufficient to assure a separate room for each species. Ideally, a separate room also should be used for each test agent under study. Cages with solid sides and bottoms and covered by filter tops must always be used when studies cannot be physically separated.

III.E.4. *Ventilation, Temperature, and Humidity* Special attention should be given to ensure that the animal facilities are properly ventilated. Each animal room should undergo 10-15 fresh-air changes per hour. The air pressure should be adjusted so that the animal rooms are slightly positive to the "dirty" corridor and negative to the "clean" one. All air must be adequately filtered before it enters or leaves the animal facilities. The temperature and humidity should be maintained at those settings that have been reported to be optimal for the animal species being used.[5] An automatic recording and alert system should be used to monitor the ambient conditions in each animal room.

III.E.5. *Emergency Power* An emergency power source should be available as a backup to the primary system. Its generating capacity should be sufficient to power the animal facilities' air-condition and light systems.

III.F. *Animal Identification* Whenever individual animal data are to be routinely recorded, each animal should be marked at the outset of the study by a standard method of identification; e.g., ear notching, toe clipping, or tagging.

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5. The CBP recommends a temperature of 74°F ±2°F (23.3°C ±1.1°C) and a relative humidity of 40% ±5% be maintained in rat and mouse rooms.

III.G. *Cages/Racks* Plastic or stainless-steel cages with solid sides and bottoms should be used, especially for studies in which the test agent may be scattered or excreted. Wire-mesh cages may be required for certain types of studies; e.g., inhalation. Animals housed in cages with solid sides and bottoms should be changed to a sanitized cage[6] with fresh bedding as frequently as necessary, but not less than once weekly. Wire-mesh cages should be sanitized no less than once every two weeks.

Racks may be of either the shelf or suspended-drawer type and should be sanitized either in place or moved to a wash area for cleaning. If a rack washer is not available, they should be scrubbed with a suitable detergent and hosed down under high pressure. Racks should be sanitized at least once every other week.

III.H. *Filters* When cages with solid sides and bottoms are used, nonwoven polyester fiber filters should be placed over them as a disease control and chemical containment measure. Bonnets or filter sheets should be replaced by sanitized ones at least once every two weeks.

III.I. *Feeders* Feeders that are adequately designed to prevent soiling, bridging, and scattering of the feed are acceptable when pellet-type rations are used. Although no feeder is completely satisfactory for meal feed, a hopper-type feeder that is firmly attached to the cage appears to cause the least problems. However, this type of feeder may still require daily "bumping" to dislodge bridged meal. An open, unfixed feed cup should not be used nor should the feed be placed directly onto the cage floor. A sanitized feeder[6] should be supplied at least once weekly.

III.J. *Water/Water Bottles* An adequate supply of fresh and suitably treated water should be provided *ad libitum*. Potential pathogens carried in the water should be killed or removed through appropriate treatment; e.g., sterilization, pasteurization, or filtration. Each cage should be supplied at least twice weekly with a sanitized water bottle, stopper, and sipper tube.[6] The bottles should be filled and the stoppers and sipper tubes inserted into them only outside of the animal rooms. Empty or partially full

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6. To assure destruction of pathogens, water at 180°F (82°C) should be used for a period in the rinse cycle. Sanitization of bottle stoppers and sipper tubes also may be done by either germicide treatment before washing or by boiling after washing.

water bottles should be replaced rather than refilled. When an automatic watering system is used, the valve-end should be located in such a manner as to prevent accidental flooding of the cage.[7]

III.K. *Rations* A nutritionally balanced standard laboratory feed which supports normal growth and maintenance should be used.[8] Other factors to consider in choosing a feed should include the constancy of its major ingredients and their sources, its moisture content, freshness, storage characteristics, and timely delivery. Non-nutritional intentional additives, such as antibiotics and estrogens, should be avoided. Whenever a change in diet manufacture occurs during the course of a study, both the test and associated control groups should be switched at the same time to the new feed. Periodic analyses of the feed for pesticide (chlorinated hydrocarbons, etc.), mycotoxin (aflatoxins, etc.), and industrial (polychlorinated biphenyls, etc.) contaminants are recommended. The data from such analyses should be retained and included in the final report on each test agent. Whenever practical and consistent with the disease control program, the feed should be sterilized. Care should be taken that the nutrients are not degradated or the palatability of the feed altered. Feed should be provided as often as necessary, but not less than once weekly, to assure an adequate supply of fresh rations. When a test agent is given in the diet, its stability also must be considered as a factor in determining how often the feed should be renewed (see Section V.C.). Contaminated or spoiled feed should be replaced within the same working day in which it is found.

III.L. *Bedding* Although mycotoxin-free ground corncob may be used, heat-treated hardwood chips are considered the most desirable type of bedding. Softwood chips or creosoted wood should not be used. The bedding should be sterilized. In studies in which open wire-mesh cages are used, an absorbent material that effectively collects and holds waste matter should be placed under them.

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7. A break in the primary containment barrier is created by the aperature in the cage which allows the animals to reach the valve end. Thus, before use of an automatic watering system, consideration should be given to the nature of the compounds to be tested and safety requirements.

8. Consideration should be given to the use of the *NIH Open Formula Rat and Mouse Ration* (Appendix B).

IV. SAFETY and HEALTH

IV.A. *Personnel Protection* Each test agent should be treated as a potential carcinogen. Thus, every precaution should be taken to prevent inadvertent exposure of personnel and the environment to the test agent. Each aspect of the bioassay study, from receipt of the test agent through histopathology, must be monitored and controlled. Personnel whose medical condition, e.g., depressed immune response, pregnancy, and steroid or cytotoxic drug treatment, may make them unusually susceptible to the possible harmful effects of a test agent should be excluded from any area where accidental exposure might occur. Individuals who are allergic to laboratory animals should not be exposed to them unless adequately protected and approval has been given by the medical or safety officer. Each laboratory should develop a safety and health plan for the handling of potential carcinogens. Applicable federal, state, and local regulations must be adhered to, as well as the National Cancer Institute's safety standards for research involving chemical carcinogens (Appendix C).

IV.B. *Animal Protection* Strict hygienic and disease prevention measures should be routinely practiced. Access to the animal facilities should be restricted to only those individuals essential to their operation. Personnel should receive adequate animal care and personal hygiene training and instruction as to the proper operating procedures. Personnel with respiratory infections or other disease conditions which may affect the animals' health should be excluded from the animal rooms.

IV.C. *Protective Clothing and Equipment* All personnel who may be exposed to either the animals or test agents must be adequately protected. A complete change of clean clothing should be provided daily and should include a fully fastened laboratory suit, gloves, boots, and head cover. The protective clothing should not be worn outside of the work area. An appropriate face mask or respirator should be worn as protection against dust, mists, or fumes.

V. CHEMICAL QUALITY and TREATMENT MIXTURE CONTROL

V.A. *Storage/Shelf Life* The proper method(s) for the long-term storage of each bulk test agent should be determined prior to its bioassay. A sample of the bulk test agent should be analyzed periodically during the study to ensure that it has retained its original characteristics.

V.B. *Purity* The purity of each test agent should be determined prior to its bioassay. In some instances, identification and determination of the percent of each impurity, as well as the purification of the test agent, may be desirable.

V.C. *Stability* The stability of each test agent should be determined under the same conditions in which it will be administered, e.g., in the feed and/or vehicle, and temperature and/or pH, prior to the start of the bioassay study. This analysis should be done on samples taken from newly prepared treatment mixtures and from the last of usable ones; e.g., diet mixes. The frequency at which fresh treatment mixtures are prepared may depend on the stability of the test agent under its conditions of storage and/or administration.

V.D. *Homogeneity/Concentration* When the test agent is incorporated into the feed, its homogeneity and concentration in the diet mix should be determined before the start of the bioassay study. Random samples from freshly mixed batches should be analyzed periodically during the study to ensure that the proper mixing and formulation procedures are being used.

V.E. *Treatment Mixtures* Treatment mixtures should be stored in inert, shatter-proof containers with air-tight covers. Each container should be clearly marked so that its contents can be easily and accurately identified. The storage containers should be located in a secure area that is free of congestion and with restricted access.

An inventory of each treatment mixture should be maintained on a current basis. A record also should be kept of the preparation and usage of each treatment mixture, including the dates and quantities prepared or used and the names of the responsible individuals.

VI. ANIMAL RANDOMIZATION

VI.A. *Randomization Procedure* Ideally, all animals should be within 2-3 days of the same age. The animals should be initially segregated into equal weight distribution groups and then divided into experimental groups (treatment and appropriate controls) using formal randomization methods (Appendix D). This procedure will ensure that each group will contain animals of approximately the same weight and age.

VII. PRECHRONIC TOXICOLOGY

The purpose of the prechronic toxicologic studies is to predict the maximum tolerated dose (MTD) of the test agent that can be given to the animals during the chronic study. This dose is predicted on the basis of the data collected from a 90-day subchronic study. However, when there are insufficient toxicologic data on the test agent, an acute and/or short-term repeated dose study may be needed prior to the subchronic study.

VII.A. *Acute Toxicity Study*

VII.A.1. *Need* An acute toxicity study may be necessary when data are unavailable as to the toxicity of the test agent. Its purpose is to determine the lethality of the test agent as well as to gain other information on its acute toxicity. The data collected should be used as a guideline to determine the dose range to be used in subsequent studies designed to predict the MTD.

VII.A.2. *Experimental Groups* To determine the lethality of the test agent, at least five animals (no older than young adults) of each sex and strain to be studied in the chronic phase should be used at each of the dose levels to be tested.

VII.A.3. *Route* The route of administration of the test agent should be the same one to be used in the chronic study.

VII.A.4. *Dose Levels* The lethality of the test agent can be estimated by use of a series of three of more dose levels which increase in a geometric progression; e.g., by a factor of two. For the purpose of this study, a precise lethal dose (LD) value need not be determined.

VII.A.5. *Treatment/Observation Periods* On day one of the study the animals should be administered an acute dose of the test agent. The duration of the exposure may be either short, e.g., a single injection or intubation, or continuous such as in the feed or water. Whatever the route or mode of administration, the duration of exposure should not exceed 24 hours. The test agent should be given in the same formulation or closely approximate the one to be used in the chronic study.

After treatment, the animals should be held a minimum of 14 days for observation.

VII.A.6. *Observations* It is important that all relevant clinical signs be recorded, including animal weights at the beginning and termination of the study and the time to death of each animal. An indication as to the type of lesions produced by the test agent may be obtained by necropsies on some or all of the animals.

VII.A.7. *Dose Selection* An analysis of the data should be made to estimate the dose at which 10% of the animals are killed. This dose can be found by a probit or logit analysis of the data plotted on appropriate graph paper. The dose selected should be the highest one used in subsequent studies designed to predict the MTD.

VII.B. *Repeated-Dose Study*

VII.B.1. *Need* The 14-day repeated-dose study should be done when the available toxicologic data are insufficient to determine the dose levels to be used in the subchronic study.

VII.B.2. *Experimental Groups* Five animals (no older than young adults) of each sex and strain to be studied in the chronic phase should be used at each of the dose levels to be tested. A similar number of comparable animals should be used as controls.

VII.B.3. *Route* Administration of the test agent should be by the same route to be used in the chronic study.

VII.B.4. *Dose Levels* Five dose levels should be initially tested. The upper level should be the one that is estimated to produce no more than 10% lethality following administration of a single acute dose of the test agent. The remaining levels should be fractions thereof; e.g., one-half, one-fourth, one-eighth, etc. If these fail to provide the information needed to select the dose range to be used in the subchronic phase, the study should be extended using the initial results to determine whether higher or lower doses should be further tested.

VII.B.5. *Treatment/Observation Periods* Animals should be treated daily with the test agent for a period not exceeding 14 days, after which they should be held another 24 hours before sacrifice. The exposure may be of either a short duration, e.g., a single daily injection or intubation, or continuous such as in the feed or water. The test agent should be given in the same formulation as the one to be used in the chronic study.

VII.B.6. *Observations* Daily observations should be made for clinical signs of toxic effects. Weights should be recorded at the beginning of the study and thereafter at weekly intervals. For some test agents, especially those that may produce delayed toxicity or cumulative effects, other measurements, e.g., organ function, body burden, absorption, and excretion also may provide information which is useful in predicting the proper subchronic dose levels. Animals should be necropsied and pathologic studies done in accordance with the *Bioassay Pathology Procedures* (Appendix E).

VII.B.7. *Dose Selection* A review of the data should be made to select the maximum dose to be used in the subchronic study. This dose should be the highest one that did not produce clinical signs of toxicity, pathologic lesions, or mortality.

VII.C. *Subchronic Study*

VII.C.1. *Need* The purpose of the subchronic study is to predict the toxic effects which may occur in the animals during the chronic administration of the test agent. Based on the subchronic observations, a prediction is made of the maximum tolerated dose (MTD) that can be given to the animals in the chronic study without producing unwanted side effects.

VII.C.2. *Experimental Groups* The experimental groups composing the subchronic study should include the test and appropriate control animals of each sex and strain to be used in the chronic study. Each experimental group should contain 10 animals of one sex and strain at a single dose level. The age of the animals should be the same as those to be used to start the chronic study.

VII.C.3. *Route* Administration of the test agent should be by the same route to be used in the chronic study.

VII.C.4. *Dose Levels* Five dose levels should be tested -- the highest being the one that does not produce clinical signs of toxicity, pathologic lesions, or mortality in short-term acute or repeated-dose studies. The remaining dose levels tested should be fractions thereof; e.g., one-half, one-fourth, one-eighth, etc. If these fail to bracket the range in which the MTD can be predicted, the study should be extended and the information from the initial attempt used as a guide in setting the dose levels for the next series to be tested.

VII.C.5. *Treatment/Observation Periods* The test agent should be administered to the animals for 90 days[9], according to the same treatment schedule and formulation to be used in the chronic study.[10] The amount of test agent administered or consumed by the animals should be quantitated. Although for some modes of administration, e.g., in the feed, the intake cannot be determined precisely, an estimate should be made of the amount consumed.

VII.C.6. *Observations* Daily observations should be made for clinical signs of toxic effects. For some test agents, especially those that may produce delayed toxicity or cumulative effects, other measurements, e.g., organ function, body burden, absorption, and excretion, also may provide information which is useful in predicting the proper chronic dose levels. Individual animal weights should be recorded weekly and plotted so that the weight gain of each group can be compared over the length of the study. Animals should be necropsied and pathologic studies done in accordance with the *Bioassay Pathology Procedures* (Appendix E).

VII.C.7. *MTD Determination* A MAXIMUM TOLERATED DOSE (MTD) should be selected for each sex of each strain to be used in the chronic study. The MTD is defined as the highest dose of the test agent given during the chronic study that can be predicted not to alter the animals' normal longevity from effects other than carcinogenicity. The MTD is estimated after a review of the subchronic data. Since these data may not always be easily

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9. For compounds that show delayed toxicity or cumulative effects, a treatment period of 120-150 days or longer may be needed.

10. In some instances, a short observation period following treatment may be desirable to predict the animals' recovery potential.

interpretable, a degree of judgment is often necessary in estimating the MTD. The MTD should be the highest dose that causes no more than a 10% weight decrement[11], as compared to the appropriate control groups; and does not produce mortality, clinical signs of toxicity, or pathologic lesions (other than those that may be related to a neoplastic response) that would be predicted to shorten the animal's natural life span. Other measurements (see VII.C.6.) also may be used to aid in predicting the MTD.

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11. Although a depressed weight gain is a clinical sign of toxicity, this particular effect is acceptable when estimating the MTD.

VIII. CHRONIC STUDY

VIII.A. *Need* The purpose of this study is to determine the carcinogenicity of the test agent in both sexes of two species and is designed to cover the greater part of the animals' life spans.

VIII.B. *Experimental Groups*

VIII.B.1. *Animal Groups* Each experimental group is composed of animals of the same sex and strain receiving an identical treatment. The usual experimental groups used in the chronic study are shown below.

VIII.B.1.a. Test groups - Each test group is composed of animals exposed to an identical dose level of the test agent. For example, female strain X mice exposed to a specific dose level would comprise one test group. Mice at a different dose level or of another sex or strain would comprise a separate test group.

VIII.B.1.b. Control groups - One or more different control groups may be necessary to evaluate the carcinogenicity of the test agent. The most common are:

VIII.B.1.b.i. Vehicle control groups - Animals in these groups are treated with only the vehicle in which the test agent is dissolved or dispersed.

VIII.B.1.b.ii. Untreated control groups - Animals in these groups should be identical in every respect to those in the other experimental groups except that they receive no specific treatment.

Although the above controls may be adequate, the appropriate matched control groups necessary to evaluate a test agent's carcinogenicity also may be those receiving another material or exposed to a given procedure used in its administration. Except for the specific treatment, all experimental groups should be identical in every respect, including source and conditions of maintenance.

In addition to the appropriate matched controls, located in the same room as the test groups, it also is desirable to have a group of untreated animals (colony controls) in a separate room where the likelihood of their inadvertent exposure to a test agent is reduced. Colony control groups should be maintained for each strain from each animal supplier. Ideally, colony controls should be initiated whenever a new shipment of animals is received.

VIII.B.2. *Animal Age/Group Size* At the start of the chronic study, animals should be no older than 6 weeks of age and, if possible, weanlings. Each test and appropriate control group should contain 50 animals. When several test agents are placed on study at the same time, common untreated and, where applicable, other control groups may be used. The size of such control groups should be increased by a factor equal to the square root of the number of test agents to which they are common. Thus, a control group common to 4 test agents would contain 100 animals ($100 = 50\sqrt{4}$). Common control groups should be located in the same animal room as the test groups are housed.

VIII.C. *Route* Administration of the test agent should be by a route that duplicates or closely approximates the one by which human exposure occurs.[12]

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12. When the route is oral, consideration should be given as to whether the test agent should be administered by gastric intubation, in a diet mix, or in the water. The introduction of the test agent by gastric intubation offers greater hazard control, better quantitation, the need for less test agent, fresher preparations, easier storage, and increased animal handling. Its disadvantages are that there is generally less than maximum intake of the test agent, a solvent is usually required, mortality may be increased, and animals need to be closely matched by weight. Diet mix offers a greater total intake of the test agent and may more closely simulate the mode of human exposure. Its disadvantages are that contamination may be difficult to control, the homogeneity of the mix is not assured, decomposition of the test agent may occur during storage or while in the feeder, the palatability of the feed may be affected, and the quantity ingested by each animal may vary. The advantages and disadvantages of giving the test agent in the water are similar to those encountered when the diet mix is used as the mode of administration.

VIII.D. *Dose Levels* At least two dose levels of the test agent should be studied in each sex of each strain. The highest dose should be the MTD, as predicted from the subchronic data, and the remaining level(s) a fraction thereof.[13] In instances in which the test agent does not cause signs of toxicity at levels up to 5% (50,000 ppm) in the diet, it is recommended that this concentration not be exceeded except under special circumstances; e.g., when the test agent is used as a major human dietary constituent. If clinical signs of toxic effects appear during the course of the chronic study, appropriate action must be taken to ensure maximum animal survival. The type of action will depend upon the objective of the study and may include lowering the dose, discontinuing treatment, or changing the dose regimen. Although the doses may need to be adjusted downward during the course of the study, they should never be raised. Frequent and competent animal surveillance must be constantly stressed.

VIII.E. *Treatment Period* The test agent should be given for a sufficient time to produce a maximum response in the animals. Although prospectively this period can only be estimated, usually the greater part of the animal's life span is considered desirable.[14] To reduce the possibility of exposing the necropsy technicians to the test agent or its metabolite(s), treatment of the animals should be discontinued one week prior to their scheduled sacrifice date. Except when the test agent is given in the drinking water or diet, the frequency of treatment will depend upon the route of administration and mode of human exposure. When the test agent is given in the water or in the feed as part of the diet mix, it should be administered on a 7-day-a-week basis. The amount of test agent administered or consumed by the animals should be quantitated. Although for some modes of administration, e.g., in the feed, the intake cannot be determined precisely, an estimate should be made of the amount consumed.

VIII.F. *Observation Period* It may be desirable to hold the animals for an additional period after cessation of treatment. This period of time may allow the tumors induced by the test agent to

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13. The CBP usually uses the MTD and either the MTD/2 or MTD/4. Thus, when both sexes of two species are used, as many as 4 different MTD's could be studied.

14. The CBP usually requires a minimum treatment period of 24 months. At the end of this time an evaluation is made as to whether the treatment period should be extended or terminated.

show a biological potential, e.g., invasion, metastases, or transplantability, that is not apparent at the end of the treatment period. Also during this time other treatment-related lesions may regress. An observation period of 3 to 6 months is usually considered to be sufficient.

VIII.G. *Observations* High priority should be given to frequent and competent animal surveillance. A viability check of every animal should be made once in the morning and once in the late afternoon on a 7-day-a-week basis. Animals in poor health or with life-threatening tumors should be isolated. Those whose condition makes it unlikely that they will survive another 24 hours should be sacrificed. Every animal should be palpated and carefully examined at least once a week.

Animal weights should be recorded on day one of the study and thereafter at monthly intervals. Ideally, weights should be recorded on an individual animal basis. The frequency of weighing should be increased to once every two weeks when either a tumor is detected in an animal from a test group or there is an apparent deterioration in the animals' health. The same weighing schedule used for the test-group animals also should be applied to their matched controls. Feed consumption should be measured on a regular basis. Usually such measurements can be conveniently made using the same schedule used to record animal weights.

In certain cases, particularly for those chemicals that persist in the animals, it may be possible and desirable to determine the distribution and concentration of the test agent or its metabolite(s) in the tissues, including blood. Such an analysis may permit a correlation to be made between the tissue concentration and the test agent's carcinogenicity.

VIII.H. *Termination* The animals in a test group should be sacrificed when either the original or adjusted termination date is reached.[15] A test group should be terminated earlier only when its cumulative mortality is sufficiently great so that prolongation of the group is no longer statistically justified. Control group animals should be sacrificed according to the original or adjusted termination date, whichever is later. Animals should be necropsied and pathologic studies done in accordance with the *Bioassay Pathology Procedures* (Appendix E).

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15. A test group is usually terminated when survival reaches 10% of the original number of animals in that group, although in some circumstances it may be earlier or later.

IX. CARCINOGEN TREATMENT GROUP

IX.A. *Need* A Carcinogen Treatment Group (Positive Control) may be desirable to determine whether the animals used to study the carcinogenicity of the test agents respond in a predictable manner to a known carcinogen. The results from a Carcinogen Treatment Group also can be useful in evaluating the carcinogenicity of the test agents. A Carcinogen Treatment Group need not be initiated concurrently with each new set of test groups but should be started at reasonable periodic intervals to monitor the animals' responsiveness.

IX.B. *Carcinogen/Dose Level* Whenever possible the chemical structure of the carcinogen should be similar to the class of test agents under study. There also should be an adequate amount of experimental data with the chosen carcinogen in the strains being used so that the tumor type(s), latent period(s), and incidence(s) can be reasonably predicted. The carcinogen should be of a sufficient potency to produce a detectable number of tumors in the exposed animals. Only a single dose level need be tested.

IX.C. *Animals/Group Size* The sexes and strains of the animals comprising the carcinogen treatment groups, and their conditions of maintenance, should be identical to those used to study the carcinogenicity of the test agents. Although fewer animals can be used with potent carcinogens than with weaker ones, each treatment group should contain at least 20 animals.

X. DATA RECORDS and REPORTS

X.A. *Required Information* It is essential that all the information and data pertaining to a bioassay study are completely and accurately recorded on a current basis. This should include all the information related to the test agent, animals, procedures, and results. Because of its importance and the scope of the effort, a plan for the collection of this information should be devised before the start of a bioassay study. As a minimum, the information recommended in the report by the *Panel on Carcinogenicity Testing of the International Union Against Cancer* (Appendix F) should be collected and be available for review during and at the end of a bioassay study.

X.B. *Methods of Collection*

X.B.1. *Logbooks* The key to a successful logbook system is ease, accuracy, and completeness in recording and retrieving data. However, when dealing with many studies and large numbers of animals, manually maintained logbooks may become bulky and difficult to manage. The preparation of reports requires the extraction, review, consolidation, and tabulation of data from the logbooks. Much of this time-consuming hand operation has been overcome with the development, by the National Cancer Institute, of a computerized system to collect, retrieve, tabulate, and analyze bioassay test data. This system is briefly described in the sections below.

X.B.2. *Carcinogenesis Bioassay Data System (CBDS)* The CBDS (Linhart, 1974) is used extensively by the CBP to collect, retrieve, tabulate, and analyze bioassay test data. It also serves to manage and monitor the status and progress of individual bioassay studies as well as to summarize the total effort of the CBP. Data input is usually through a series of forms (see pages 23 to 28) submitted to the CBP where they are processed for entry into a computer. The *Systematized Nomenclature of Pathology (SNOP)* is used to code the pathology results collected on individual animals.

As the usefulness and integrity of the data output are only as good as the accuracy of the data input, it is necessary to closely monitor data before their entrance into the system. To ensure that the data technicians at the field level have the proper background, special training classes are periodically

held at the NCI.[16] After coding and prechecking, the data forms are translated into a machine-readable format and entered into the system, microfilmed, and stored as microfiche for future reference. After computer editing for errors, the data are available for selective or complete recall. Data output is available as a series of standard or special reports and tables presenting the data in the following ways:

a. bioassay studies underway within the total program or within a particular contract effort,

b. an individual bioassay study,

c. special pathology reports,

d. survival and weight curves, and

e. selected analysis of the test data.

Many of the reports are generated for use by the field investigator and other concerned individuals and may be readily available. Other reports are for internal use or to support the activities of other Governmental agencies and usually have a limited distribution.

X.C. *Reports* In view of the possible use of bioassay test results in establishing public policy, the general release of such data is normally done only after the data have been completely confirmed, verified (including pathology review), and statistically analyzed. The final publication is made through the issuance of a Technical Report. Other reports of a less complete nature may precede this final report.

X.C.1. *Chemicals on Carcinogenesis Bioassay* This report lists all the chemicals under test for carcinogenicity by the CBP.[17]

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16. For information regarding these classes or any phase of the data system, write Carcinogenesis Bioassay Data system, P.O. Box I, Bethesda, Maryland 20014.

17. This report may be requested from the Chief, Carcinogen Bioassay and Program Resources Branch, Landow Building, Room C319, National Cancer Institute, Bethesda, Maryland 20014.

BATCH CHEMICAL DATA RECORD

KEYPUNCH OPERATOR: *Columns 2 through 15 apply to each card on this page.*

CONTRACT NUMBER	CHEMICAL NUMBER	BATCH NUMBER
2 - 7	8-13	14 - 18

ITEM			CARD COL.
SECTION I			
1. Chemical Common Name			
2. Name of Manufacturer	FOR OFFICE USE (Do Not Code)		25 - 27
3. Chemical Name Used in Catalog			
4. Manufacturer's Lot Number of Batch			
5. Is batch labeled? (e.g. isotope)	1 - Yes* 2 - No		28
6. Is there a reserve supply of batch?	1 - Yes 2 - No		29
7. Is batch purity tested?	1 - Yes *(Results of analysis)** 2 - No		30
8. Physical State of Chemical Batch			31
1 - Solid *(Indicate melting range in Item 9)* 2 - Liquid *(Indicate boiling range in Item 9)* 3 - Gel *(Indicate liquification range in Item 9)* 4 - Gas *(Skip Item No. 9)*			

ITEM		CARD COL.
9. Critical Temperature Range *(Degrees Centigrade)*	TO	32 - 39
10. How was the physical property obtained?	1 - Observed 2 - Literature 3 - Manufacturer's Specifications	40
SECTION II		
The following information should be provided only if available. Enter "1" in appropriate boxes and report data in Comment area.		
11. Date Received from Supplier*		41
12. Date of Manufacture and Original Storage by Manufacturer*		42
13. Methods of Preparation or Synthesis*		43
14. Special Treatment Applied After Receipt *(e.g. purification methods)**		44
CARD NUMBER	8	80

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

MIH-1624-2
Rev. 11-71

PREPARATION DATA RECORD

KEY PUNCH OPERATOR: *Columns 2 through 11 apply to each card on this page.*

CONTRACT NUMBER	PREPARATION NUMBER	
2 - 7	8	9 - 11
	P	

1. Composition of Preparation *(If method of preparation is other than simple solution, describe the process and the physical state of the resultant preparation)**

a. CHEMICAL NAME	b. CHEMICAL BATCH USED	c. NCI CHEMICAL NUMBER	d. % CONCENTRATION	e. EXPONENT	f. UNIT CODE 1/	CARD NUMBER
		25 - 30	31 - 34	35 X 10 -	36	
		37 - 42	43 - 46	47 X 10 -	48	
		49 - 54	55 - 58	59 X 10 -	60	80 1
		25 - 30	31 - 34	35 X 10 -	36	
		37 - 42	43 - 46	47 X 10 -	48	
		49 - 54	55 - 58	59 X 10 -	60	80 2

1/ UNIT CODES: 1 - Weight/Weight 2 - Weight/Volume 3 - Volume/Volume

ITEM		CARD COL.
2a. How often prepared?	1 - Once 2 - Fresh for each treatment 9 - Other*	25
b. If Once, Indicate Date of Preparation	Month Day Year	26 - 31
3. Mode of Storage	1 - Open shelf - dark 2 - Open shelf - light 3 - Refrigerator or freezer *(indicate temperature)** 4 - Not stored 9 - Other*	32
4a. Chemical Stability	1 - Stable 2 - Unstable 9 - Unknown	33
b. If Unstable, indicate length of stability as stored	Days	34 - 36

ITEM		CARD COL.
5. pH *(Aqueous systems only)*	.	37 - 39
6. Particle size *(Where applicable)*		
MASS DIAMETER a. Median	.	40 - 43
b. Standard deviation	.	44 - 47
COUNT DIAMETER c. Median	.	48 - 51
d. Standard deviation	.	52 - 55
CARD NUMBER	3	80

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

MIH-1624-4
Rev. 11-71

ENVIRONMENTAL DATA RECORD

KEYPUNCH OPERATOR: Columns 2 through 10 apply to each card on this page.

CONTRACT NUMBER (2 - 7)	ENVIRONMENT NUMBER (8)	(9 - 10)
	A	

ITEM	CARD COL.
1. Does information on this form apply to all animals in this contract? 1 - Yes 2 - No *(If No, complete 2a or b below)*	25
2a. If it applies only to all animals within particular colonies list colony numbers	26 - 28 / 29 - 30 - 31 / 32 - 33 - 34 / 35 - 36 - 37
b. If it applies only to all animals within particular groups list group numbers	
If groups are sequentially numbered, list highest and lowest numbers ONLY: COLONY (38 - 39 - 40) GROUP NUMBER (41 - 44) THROUGH (45 - 46 - 47) GROUP NUMBER (48 - 51) *(Inclusive)*	
OR If group numbers are not sequentially numbered list each number: COLONY (52 - 53 - 54) GROUP NUMBER (55 - 58); (59 - 60 - 61) (62 - 65); (66 - 67 - 68) (69 - 72); (73 - 74 - 75) (76 - 79)	
CARD NUMBER [1]	80

ITEM		CARD COL.
3. Will information on this form apply for the entire period of the experiment? 1 - Yes 2 - No*	Feed	25
	Bedding	26
	Cages	27
	Lighting	28
	Water	29
	Room Air	30
4. Frequency of Changing and Sanitizing 1/	Cages	31
	Cage lids	32
	Water bottles	33
	Feed hoppers	34
	Bedding	35
1/KEY TO CODES	1 - Never; 2 - 1x/month; 3 - 1x/week; 4 - 2x/week; 5 - 3x/week; 6 - Daily; 9 - Other*	

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

ENVIRONMENTAL DATA RECORD (Continued)

KEYPUNCH OPERATOR: Columns 2 through 10 apply to each card on this page.

CONTRACT NUMBER (2 - 7)	ENVIRONMENT NUMBER (8)	(9 - 10)
	A	

ITEM	CARD COL.
SECTION I - FEED INFORMATION	
5. Is feed commercial? 1 - Yes 2 - No *(Indicate composition)** *(If Yes, answer items 6 through 8; else SKIP to item 9)*	38
6. Name of Feed	37 - 56
CARD NUMBER [2]	80
7. Name of Feed Manufacturer	25 - 54
8. Name of Feed Supplier *(If different from manufacturer)*	
9. Was analysis of feed made? *(If Yes, specify results of analysis)** 1 - No 2 - Yes - by manufacturer 3 - Yes - by other than manufacturer	55
10. Feed Consumption 1 - Ad lib 2 - Measured 9 - Other*	56
11. In what form is feed given to animals? 1 - Pellet 2 - Powder 3 - Semi-solid 4 - Liquid 9 - Other*	57
12. How is feed given to animals? 1 - Cage lid 2 - Separate pan or hopper 3 - Bottom of cage 9 - Other*	58

ITEM		CARD COL.
SECTION II - BEDDING INFORMATION		
13. Is bedding utilized? 1 - Yes 2 - No *(If Yes, complete items 14 and 15; else SKIP to item 16)*		59
14. Name of Bedding		60 - 79
CARD NUMBER [3]		80
15. Name of Bedding Manufacturer		25 - 54
16. Type of Bedding Material	1 - Wood chips *(what type)**; 2 - Wood shavings *(what type)**; 3 - Corn cobs; 9 - Other*	55
17. Was bedding sterilized?	1 - Yes - *(what method)**; 2 - No	56
SECTION III - CAGE INFORMATION		
18. Cage Dimensions *(inches)*	a. Length	57 - 59
	b. Width	60 - 62
	c. Height	63 - 65
19. Number of Cages/100 Square Feet of Floor Space		66 - 68
20. Are cages rotated in room?	1 - No; 2 - Yes - positioned at random; 3 - Yes - other method*	69
CARD NUMBER [4]		80

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

ENVIRONMENTAL DATA RECORD (Continued)

KEYPUNCH OPERATOR: Columns 2 through 10 apply to each card on this page.

CONTRACT NUMBER (2 - 7) | ENVIRONMENT NUMBER (8 - 9 - 10) | A

ITEM		CARD COL.
21. Name of Cage Manufacturer		23 - 54
22. Cage Material	1 - Polycarbonate 2 - Polypropylene 8 - Metal - (Indicate what type)* 9 - Other*	55
23. Does cage have air filter?	1 - Yes 2 - No	56
24. Construction of Cage Floor	1 - Solid 2 - Perforated 3 - Wood slats 9 - Other*	57
25. What method of cage sanitation is used?	1 - Autoclave 2 - Steam clean 3 - Total replacement 4 - Chemical* 9 - Other*	58

SECTION IV - LIGHTING INFORMATION

26. Indicate the Type of Light Used and Time of Exposure

(More than one answer may be applicable. Complete all that apply.)

SOURCE	EXPOSURE (Hours per day)	CARD COL.
1 - Natural		59 - 60
2 - Tungsten		61 - 62
3 - Fluorescent		63 - 64
4 - Ultraviolet		65 - 66
9 - Other*		67 - 68

COMMENTS: Refer to Item No. when making comments.

ITEM			CARD COL.
SECTION V - WATER INFORMATION			
27. Water Source	1 - Tap 2 - Distilled 9 - Other*		69
28. Water Distribution	1 - Glass bottles 2 - Plastic bottles 3 - Automatic watering 9 - Other*		70
29. Water Consumption	1 - Ad lib 2 - Measured 9 - Other*		71
30. Chlorine Content of Water When Bottles Filled	(PPM)	.	72 - 74
31. Fluoride Content of Water	(PPM)	.	75 - 77
32. What kind of water analysis was made?	1 - Chemical 2 - Bacteriological 3 - Both chemical and bacteriological 4 - None		78
33. Have you introduced additives into the water?	1 - Yes 2 - No		79
CARD NUMBER		5	80
SECTION VI - ROOM AIR INFORMATION			
34. What is the relative humidity range? (Percent)	FROM ___ TO ___		25 - 28
35. What is the temperature range? (degrees centigrade)	FROM ___ TO ___		29 - 32
36. Number of Changes Per Hour of Room Air			33 - 34
37. Is room air filtered?	1 - Yes - (specify filter type)* 2 - No		35
38. Communication Between Animal Rooms	1 - Open 2 - Partial 3 - None		36
CARD NUMBER		6	80

* COMMENTS REQUIRED

NIH-1624-5
Rev. 12-71

COLONY DATA RECORD

KEYPUNCH OPERATOR: Columns 2 through 10 apply to each card on this page.

CONTRACT NUMBER (2 - 7) | COLONY NUMBER (8 - 9 - 10) | L

ITEM		CARD COL.
1. Animal Species		28 - 34
2. Type of Breeding	1 - Inbred 2 - Random 3 - Hybrid 9 - Other*	35
Answer Item 3 OR 4.		
3. If Breeding Was NOT Hybrid Indicate STOCK OR STRAIN		36 - 55
4. If Breeding Was Hybrid Indicate		
a. MALE PARENT Stock or Strain		36 - 55
b. FEMALE PARENT Stock or Strain		56 - 75
CARD NUMBER	1	80

ITEM			CARD COL.
5. Source of Animal Supply	1 - Own Breeding Colony (Provide history)* 2 - Outside Source (Commercial, etc.)		25
6. If Outside Source, Name of Supplier			26 - 40
7. Age of Animals When Obtained from Supplier (If in utero, enter zero)	Days		41 - 43
8. Experiment Disease Control (Place a "1" after those which apply)			
	Conventional		44
	SPF (List pathogens)*		45
	Defined Microflora (List organisms)*		46
	Germ-free		47
	Vaccinated (List organisms)*		48
	Other*		49
9. Did the same disease controls as above apply in supplier's breeding colony?	1 - Yes 2 - No*		50
CARD NUMBER		2	80

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

NIH-1624-6
Rev. 3-71

ANIMAL GROUP DATA RECORD

KEYPUNCH OPERATOR: Columns 2 through 14 apply to each card on this page.

CONTRACT NUMBER (2 - 7)	ANIMAL GROUP NUMBER COLONY (8 - 9)	(10)	GROUP (11 - 14)
		S	

SECTION I - DESCRIPTIVE DATA

ITEM		CARD COL.
1. Number of Animals in the Group		25 - 26
2. Identification Numbers of the Cages Occupied by the Group (sequential cage numbering required)	(lowest number) Through (highest number)	29 - 31 32 - 34
3. Sex of Animals	1 - Males; 2 - Females; 0 - Other*	35
4. Median Date of Birth	Month Day Year	36 - 41
5. Range of Birth Dates	(days)	42 - 44
6. Room Designation		45 - 47
7. Method of Animal Selection	1 - Random - casual; 2 - Random - systematized; 3 - Litter relationship considered*; 0 - Other*	48
8. Special Treatment (Place a "1" after those which apply)	Surgery*	49
	Anesthesia*	50
	Diet Change*	51
	Other*	52
9. Group Role in Experiment (If answer is Test, complete remainder of this form, else complete this Page ONLY.)	1 - Test; 2 - Vehicle Control; 3 - Positive Control; 4 - Untreated Control	53
CARD NUMBER	1	80

SECTION II - TREATMENT REGIMEN

NOTE: For 1, 2, and 3, see appropriate keys in Manual.

10. Preparation Number	11. Age at First Dose (In Days)	12. Route of Administration 1/	13. Amount of Preparation Per Dose (For exposure, indicate length of time)	14. Units of Dose Measurement 2/	15. Frequency of Treatment 3/	16. Duration of Treatment (Number of days, or spell out "LIFE" if life)	CARD NO.
25 - 27	28 - 30	31 - 32	33 - 36	37 - 38	39	40 - 43	80
							2
							3
							4
							5

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

ANIMAL GROUP DATA RECORD (Continued)

KEYPUNCH OPERATOR: Columns 2 through 14 apply to each card on this page.

CONTRACT NUMBER (2 - 7)	ANIMAL GROUP NUMBER COLONY (8 - 9)	(10)	GROUP (11 - 14)
		S	

▶ Complete this page ONLY if the answer to Item Number 9, Page 1, was "TEST".

ITEM		CARD COL.
17. Major Compounds in Treatment	a. NAME / b. CHEMICAL NUMBER	
		25 - 30
		31 - 36
		37 - 42
18. Did you previously list ALL control groups and the necropsy checklist for this test group? 1 - Yes 2 - No		43
19. 1-Yes (Write the test group number here and disregard the rest of this form.) 2-No (Complete rest of this form.)		44 - 47

20. Provide identification numbers of associated control groups. If group numbers are sequential, enter inclusive numbers in Schedule A below; if not sequential, use Schedule B.

SCHEDULE A

				CARD NO.
VEHICLE	48 - 51	Through	52 - 55	
POSITIVE	56 - 59	Through	60 - 63	
UNTREATED	64 - 67	Through	68 - 71	80
				6

SCHEDULE B

VEHICLE	POSITIVE	UNTREATED	CARD NO.
25 - 28	29 - 32	33 - 36	
37 - 40	41 - 44	45 - 48	
49 - 52	53 - 56	57 - 60	80
			7
25 - 28	29 - 32	33 - 36	
37 - 40	41 - 44	45 - 48	
49 - 52	53 - 56	57 - 60	80
			8

ITEM		CARD COL.
21. Organs or Tissues to be Routinely Examined at Necropsy	1 - Not examined; 2 - Gross pathology examination only; 3 - Both gross and histopathology examination	
Skin		25
Mouth		26
Nasal Cavities		27
Subcutaneous Tissue		28
Breasts		29
Muscles		30
Bones		31
Brain		32
Pituitary Gland		33
Spinal Cord		34
Ear Duct		35
Salivary Gland		36
Thymus		37
Thyroid Glands		38
Lymph Glands		39
Bone Marrow		40
Blood Smear		41
Spleen		42
Heart		43
Lungs, Bronchi		44
Trachea/Larynx		45
Kidneys		46
Bladder		47
Ureter/Urethra		48
Uterus/Prostate		49
Ovaries/Testis		50
Adrenal Glands		51
Esophagus		52
Stomach		53
Intestinal Tract		54
Pancreas		55
Liver		56
Gall Bladder/Bile Ducts		57
Other*		58
CARD NUMBER	9	80

COMMENTS: Refer to Item No. when making comments.

* COMMENT REQUIRED

GROUP OBSERVATION RECORD

KEYPUNCH OPERATOR:

Columns 2 through 10 apply to each card on this page.

CONTRACT NUMBER	COLONY		CARD CODE
2-7	8-9	10	80
		S	G

ANIMAL GROUP NUMBER	1. OBSERVATION DATE			2. NUMBER OF SURVIVORS TO DATE	3. TOTAL NET ANIMAL WEIGHT (Gms)	COUNTS			7. INCLUDE DESCRIPTION OF TUMORS, INTERCURRENT DISEASES, AND OTHER OBSERVATIONS IN AREA PROVIDED
	Month	Day	Year			4. SURVIVORS BEARING PALPABLE TUMORS	5. TOTAL PALPABLE TUMORS	6. FOOD CONSUMED IN PREVIOUS WEEKS (Gms)	
GROUP									
11-14	33-38			39-41	42-46	47-49	50-52	53-56	

DESCRIPTION

DESCRIPTION

DESCRIPTION

DESCRIPTION

DESCRIPTION

DESCRIPTION

NIH-1624-9
Rev. 10-72

GPO 888-470

CAGE OBSERVATION RECORD

KEYPUNCH OPERATOR:

Columns 2 through 15 and 80 apply to each card on this page.

CONTRACT NUMBER	ANIMAL GROUP NUMBER			CARD CODE
	COLONY		GROUP	
2 - 7	8 - 9	10	11 - 14	80
		S		C

CAGE NUMBER	1. OBSERVATION DATE			2. NUMBER OF SURVIVORS TO DATE	3. TOTAL NET ANIMAL WEIGHT (Gm)	COUNTS			7. INCLUDE DESCRIPTION OF TUMORS, INTERCURRENT DISEASES, AND OTHER OBSERVATIONS IN AREA PROVIDED
	Month	Day	Year			4. Survivors bearing palpable tumors	5. Total palpable tumors	6. Total carcinomas (Skin studies)	
15 16 - 18	33 - 38			39 - 41	42 - 45	46 - 48	49 - 51	52 - 54	

C DESCRIPTION

C DESCRIPTION

C DESCRIPTION

C DESCRIPTION

C DESCRIPTION

C DESCRIPTION

NIH-1624-3
Rev. 7-72

INDIVIDUAL ANIMAL DATA RECORD

(Columns 2–18 apply to each card punched from this form.)

INDIVIDUAL ANIMAL IDENTIFIER								
CONTRACT NUMBER		GROUP NUMBER				INDIVIDUAL NUMBER		
		COLONY		GROUP				
2–7		8–9	10	11–14		15	16–18	
			S			I		

1. LITTER NO. (If Litter Mates are Followed)	2. BODY WEIGHT AT DEATH (grams)	3. CONTRACTORS HISTOLOGY NUMBER	4. DATE OF DEATH			5. AGE AT DEATH (Weeks)	6. HOURS FROM DEATH UNTIL NECROPSY	7. DISPOSITION 1]	8. CONDITION 2]	CARD CODE
			Month	Day	Year					
25–27	28–32	33–38	39–44			45–47	48–49	50	51	80
										J

9. SPECIES
1. Mouse ☐
2. Rat ☐
3. Other ☐ (Specify) _____

1] DISPOSITION CODES
A. Scheduled Sacrifice
B. Moribund Sacrifice
C. Natural Death
D. Accidentally Killed
E. Missing
X. Other*

2] CONDITION CODES
A. Fresh
B. Refrigerated
C. Cannibalized
D. Decomposed
X. Other*

10. GROSS OBSERVATIONS

11. TISSUES FIXED: (Place a 1 by Tissues Fixed for Preservation)

12. TISSUES EXAMINED MICROSCOPICALLY (Place a 1 by Tissues Examined)

	TISSUES		CARD COLUMN
	Tissue Masses		25
	Regional Lymph Node		26
	Blood Smear		27
	Skin		28
	Mandibular Lymph Node		29
	Mammary Gland		30
	Salivary Gland		31
	Thigh Muscle		32
	Sciatic Nerve		33
	Bone Marrow		34
	Costochondral Junction,Rib		35
	Thymus		36
	Larynx		37
	Trachea		38
	Lungs and Bronchi		39
	Heart		40
	Thyroids		41
	Parathyroids		42
	Esophagus		43
	Stomach		44
	Duodenum		45
	Jejunum		46
	Ileum		47
	Colon		48
	Rectum		49
	Mesenteric Lymph Node		50
	Liver		51
	Gall Bladder		52
	Pancreas		53
	Spleen		54
	Kidneys		55
	Adrenals		56
	Bladder		57
	Seminal Vesicles		58
	Prostate		59
	Testes		60
	Ovaries		61
	Uterus		62
	Nasal Cavity		63
	Brain		64
	Pituitary		65
	Eyes		66
	External and Middle Ear		67
	Spinal Cord		68
	Other*		69
	Card Code	K	80

COMMENTS (Refer to Item No. When Making Comments)

*COMMENTS REQUIRED.

SIGNATURE OF PROSECTOR

INDIVIDUAL ANIMAL DATA RECORD

(Columns 2–18 apply to each card punched from this form.)

INDIVIDUAL ANIMAL IDENTIFIER								
CONTRACT NUMBER		GROUP NUMBER				INDIVIDUAL NUMBER		
		COLONY		GROUP				
2–7		8–9	10	11–14		15	16–18	
			S			I		

13. CONTRACTORS HISTOLOGY NUMBER

14. HISTOLOGIC OBSERVATIONS

15. ORGAN AND DIAGNOSES	DO NOT CODE		
	25–26	27–35	
	36–37	38–46	
	47–48	49–57	80
			2
	25–26	27–35	
	36–37	38–46	
	47–48	49–57	80
			3

SIGNATURE OF PATHOLOGIST

X.C.2. *Bioassay Information Sheet* This is an interim report detailing the experimental design, expected completion dates, and other information relating to the bioassay of a particular chemical. Preliminary results or conclusions are not included unless they appear to be unequivocal.

X.C.3. *Technical Report* The results from each bioassay study of a particular chemical are fully documented in this report. It contains all of the information recommended in the report by the *Panel on Carcinogenicity Testing of the International Union Against Cancer* (Appendix F) and is prepared jointly by the field investigator and the NCI staff. The results are tabulated in a chronological sequence so that each animal is easily followed. This includes time and mode of death and the associated histopathology. In addition to the "raw" data, the Technical Report contains a narrative of the experimental design, conduct of the study, pathology results, summary tables of the data, statistical analyses, discussion, and conclusions. The Technical Report provides a full documentation on the bioassay study of a particular test agent and is available to the public.

X.C.4. *Open Reports* These reports usually take the form of journal articles or papers presented at scientific meetings. After the bioassay test results have been appropriately analyzed, evaluated, and approved, the CBP encourages the dissemination of such reports on every chemical tested under its aegis.

APPENDIX A

ANIMAL CAGE
WEIGHT-SPACE SPECIFICATIONS

The weight-space specifications, shown below, conform with those recommended in the *Guide for the Care and Use of Laboratory Animals*.

SPECIES	WEIGHT	FLOOR AREA/ANIMAL (SQUARE)		HEIGHT*	
	(gm)	(cm)	(in)	(cm)	(in)
Mouse	Up to 10	39	(6)	12.7	(5)
	10-15	52	(8)	12.7	(5)
	16-25	77	(12)	12.7	(5)
	Over 25	97	(15)	12.7	(5)
Rat	Up to 100	110	(17)	17.8	(7)
	100-200	148	(23)	17.8	(7)
	201-300	187	(29)	17.8	(7)
	Over 300	258	(40)	17.8	(7)
Hamster	Up to 60	64.5	(10)	15.2	(6)
	60-80	83.9	(13)	15.2	(6)
	81-100	103.2	(16)	15.2	(6)
	Over 100	122.6	(19)	15.2	(6)
Guinea pig	Up to 250	277	(43)	17.8	(7)
	250-350	374	(58)	17.8	(7)
	Over 350	652	(101)	17.8	(7)

*Height means from the resting floor to the cage top.

APPENDIX B

NIH OPEN FORMULA RAT AND MOUSE RATION

This specification (adapted from NIH-11-133c, Jan. 2, 1973) is for an open formula laboratory rat and mouse ration which is void of any feed additives containing antibiotics or estrogenic activity. It should be noted that the optimum balance of dietary constituents for animals in carcinogen bioassay studies has not been clearly defined. Thus, deviations from this specification may sometimes be acceptable.

Materials - Material shall be as specified below:

Ingredient	Percentage by Weight
Dried skim milk	5.00
Fish meal (60% protein)	10.00
Soybean meal (49% protein)	12.00
Alfalfa meal (dehydrated 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Wheat middlings	10.00
Brewer's dried yeast	2.00
Dry molasses	1.50
Soy oil	2.50
Salt	0.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Pre-mixes	0.25
	100.00

Ingredients should be ground to pass through a U.S. Standard Screen No. 16 before mixing.

Vitamin Fortification Per Ton (2,000 lbs.) of Finished Product:

Vitamin	Amount	Source
A	5,500,000.0 I.U.	Stabilized vitamin A palmitate
D_3	4,600,000.0 I.U.	
K	2.8 g.	Menadione sodium
A-tocopheryl acetate	20,000.0 I.U.	
Choline	560.0 g.	Choline chloride
Folic acid	2.2 g.	
Niacin	30.0 g.	
d Pantothenic acid	18.0 g.	Calcium pantothenate
Riboflavin	3.4 g.	
Thiamine	10.0 g.	
B_{12}	4000.0 mcg.	
Pyridoxine	1.7 g.	
Biotin	140.0 mg.	

Mineral Fortification Per Ton (2,000 lbs.) of Finished Product:

Mineral	Amount (g)	Source
Cobalt	0.4	Cobalt carbonate
Copper	4.0	Copper sulfate
Iron	120.0	Iron sulfate
Manganese	60.0	Manganous oxide
Zinc	16.0	Zinc oxide
Iodine	1.4	Potassium iodate

These concentrations of vitamins and minerals shall be added to the ration via two separate (vitamin and mineral) premixes with one of the major ingredients as a carrier. The amount of this ingredient used in the final formulation may be adjusted so the total amount of ingredients will equal 100%. Vitamin and mineral concentrations in the premixes shall be made so a minimum of one pound of each premix is required per ton of finished product. In the case of the mineral fortification, the actual amount of each element required is specified. Therefore, the amount of each compound used in the premix shall be adjusted according to its mineral concentration.

Microanalysis - The total calculated concentration of nutrients in the ration from ingredients and from the fortifications at the time of manufacture shall be as follows:

Crude protein	%	Minimum	23.5
Crude fat	%	Minimum	5.0
Crude fiber	%	Maximum	4.5
Ash	%	Maximum	7.0

Amino acids

(% of total diet)	Minimum
Arginine	1.25
Lysine	1.20
Methionine	.50
Cystine	.35
Tryptophan	.25
Glycine	1.10
Histidine	.50
Leucine	1.80
Isoleucine	1.10
Phenylalanine	1.10
Tyrosine	.75
Threonine	.90
Valine	1.20

Minerals

Calcium	%	Minimum	1.20
Phosphorous	%	"	.95
Potassium	%	"	.80
Sodium	%	"	.33
Magnesium	%	"	.15
Iron	PPM	"	250.00
Zinc	PPM	"	45.00
Manganese	PPM	"	100.00
Copper	PPM	"	15.00
Cobalt	PPM	"	0.70
Iodine	PPM	"	1.80

Vitamins

Vitamin A	IU/g	Minimum	15.0
Vitamin D	IU/g	"	4.0
Alpha-tocopherol	PPM	"	35.0
Thiamine	PPM	"	14.0
Riboflavin	PPM	"	7.0
Niacin	PPM	"	80.0
Pantothenic acid	PPM	"	20.0
Choline	PPM	"	2000.0
Pyridoxine	PPM	"	10.0
Folic acid	PPM	"	4.0
Biotin	PPM	"	0.15
Vitamin B_{12}	Mcg/lb	"	12.0
Vitamin K	PPM	"	3.0

Approximate Analysis - Analysis for nutrient content of both ingredients and the finished product shall be conducted in accordance with the procedures of the *Association of Official Agricultural Chemists* (1965). All nutrient contents shall be expressed as a percentage by weight on an air-dry basis.

Ingredient Standards - Ingredients used in the manufacture of this ration will not be contaminated with any more than 3% of foreign materials such as other grains, weed seeds, chaff, etc. Nor will any mold, must, or insect/rodent infestation be allowed. The average minimum nutrient concentrations of ingredients used in the manufacture of this ration shall be equal to the values published in the National Academy of Sciences Publication 1684, *United States - Canadian Tables of Feed Composition.*

Form - The finished product may take the following forms:

1. Oval shape pellets 5/8"-3/4" wide; 3/8"-7/16" thick; 1"-1 3/8" long
2. Oval shape pellets 5/8"-3/4" wide; 3/8"-7/16" thick; 3/8" to 1" long
3. Meal form

Special Assays - The product covered by this specification is subject to estrogen assays in accordance with the latest issue of National Institutes of Health Standard No. 2. Estrogen assays shall be initiated by the laboratory once every two weeks. If at any time the

estrogen activity in a batch* of product is found to exceed 4 ppb, such a batch shall be rejected as unsuitable. Periodic analyses of the product also should be made for pesticide, mycotoxin, industrial, and other contaminants. The data from these analyses should be made available to the users of the product.

Feed Additives and Processing Restrictions - The product shall contain no antibiotics or estrogen additives of any kind. All milling and warehousing conditions and/or restrictions, as specified in the latest issue of the National Institutes of Health Standard No. 1, apply to the feed covered by this specification. The product shall not be altered in any manner so as to affect the final nutrient content.

Marking - Each bag shall be marked (either on the bag or via green tag) with the name of the product, the name of the manufacturer, the net weight, the ingredients, the guaranteed analysis of its contents, the date (month, day, and year) the manufacturing process was completed, and the batch number under which it was processed.

Packaging - The finished product shall be packaged in commercially acceptable 3-ply laminated paper bags, 50 lbs. per bag. The paper bags shall be closed by sewing in a manner that will ensure the delivery of uncontaminated animal feed.

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* For the purpose of this Standard, a "batch" is defined as one continuous production run which may or may not consist of several small batches.

APPENDIX C

SAFETY STANDARDS FOR RESEARCH INVOLVING CHEMICAL CARCINOGENS

The safety standards provided in this appendix have been adapted from the *National Cancer Institute Safety Standards for Research Involving Chemical Carcinogens* (DHEW Publication No. NIH-76-900). These were developed as interim standards for use by personnel employed at the National Cancer Institute (NCI). It is intended that they will be superceded by permanent DHEW safety standards as they become effective. The standards contained herein are recommended for use by all laboratories involved in research with known, potential, or suspect carcinogens.

I. POLICY

The NCI has established a policy that planning and implementation of control practices for the prevention of occupationally-acquired cancer and for the protection of the general environment shall be included in all cancer research programs.

II. INTRODUCTION

The NCI Safety Standards set forth general safety principles that are to be followed in the handling, storage, and disposal of chemical carcinogens. These Standards have been prepared to protect laboratory workers and their experiments from inadvertent exposure to chemical carcinogens, as well as to minimize the hazard to the community. They include work control practices, environmental control techniques, and a health surveillance program. The Safety Standards are specifically designed for work with chemical carcinogens -- which are long-term hazards -- and are meant to supplement conventional safety practices, such as accident and fire prevention.

III. REGULATED CHEMICAL CARCINOGENS

Specific chemical carcinogens for which Federal regulation have been promulgated by the Department of Labor are:

A. Low Molecular Weight Aliphatic Derivatives

1. Ethylenimine
2. beta-Propiolactone
3. Vinyl chloride
4. Bis (chloromethyl) ether
5. Chloromethyl methyl ether

B. Aliphatic Nitrosamine Derivatives

1. N-Nitrosodimethylamine

C. Aromatic Amine Derivatives

1. 4-Aminodiphenyl
2. 4-Nitrobiphenyl
3. Benzidine
4. 3,3'-Dichlorobenzidine
5. 4,4'-Methylene-bis (2-chloroaniline)
6. beta-Naphthylamine
7. 2-Acetylaminofluorene

D. Aromatic Azo Derivatives

1. 4-Dimethylaminoazobenzene

IV. CARCINOGEN SAFETY STANDARDS

IV.A. Responsibility

IV.A.1. Office of Research Safety, NCI

The Office of Research Safety shall administer the policy of the NCI for the prevention of occupationally-acquired cancer and for the protection of the general environment. The Office is responsible for: (1) developing standards that are necessary to carry out this policy; (2) assisting program managers and laboratory supervisors in implementing these Standards; (3) coordinating the safety activities of the laboratories of the NCI; (4) developing resources to facilitate the implementation of these Standards; (5) directing the investigation of incidents that result in personnel exposures to chemical carcinogens; (6) maintaining safety records; (7) conducting safety inspections to determine compliance; and (8) ensuring compliance through appropriate administrative channels.

IV.A.2. Laboratory Supervisors

Laboratory supervisors are responsible for training their subordinates in safe practices, for correcting work errors and conditions that may result in personal injury, including exposure to chemical carcinogens, and for developing a positive attitude toward safety in laboratory operations on the part of their subordinates. Laboratory supervisors investigate the circumstances surrounding each accident that results in personal injury or exposure to chemical carcinogens; initiate corrective action and recommend to management improvements that ensure maximum safety for their subordinates; and are responsible for preparing safety action plans for the research under their direction. The safety action plans shall describe: (1) the operational practices that will be used to implement these Standards; (2) the emergency procedures to be followed in the event of an accidental exposure to a chemical carcinogen; and (3) the monitoring procedures to assure the effectiveness of the operational practices. The safety action plans shall be approved by the Office of Research Safety. The laboratory supervisors shall notify the Office of Research Safety immediately on the occurrence of any accident that results in the exposure of personnel or the environment to a chemical carcinogen.

IV.A.3. Laboratory Workers

Laboratory workers are responsible for complying with oral and written safety rules, regulations, and procedures required for the performance of the assigned task. This is necessary for the protection of the laboratory workers, other fellow workers, and the public. They also are responsible for reporting to their immediate supervisor all facts pertaining to every accident resulting in personnel injury or exposure to a chemical carcinogen and any action that could result in such incidents.

IV.B. Medical Surveillance

IV.B.1. Preassignment Examinations

An appropriate preassignment physical examination shall be provided each person planning to work with chemical carcinogens. The purpose of this examination is to establish a base line against which changes can be measured and to determine whether there exist any medical or other conditions that may lead to an increased risk for the person in the work situation.

IV.B.2. Periodic Examinations

All employees working with chemical carcinogens shall be provided periodic physical examinations. The purpose of the examinations is to determine whether a change has occurred in the employees' medical state or in other relevant conditions that might lead to increased risk in the work situation. The frequency shall be dependent upon the work circumstances and the general health of the employee.

IV.B.3. Records

Medical records shall be maintained for the duration of the employee's employment by the NIH Employee Health Service. Upon termination of the employee's employment, including retirement or death, the medical records, or copies thereof, shall be transmitted to the Office of Research Safety which will forward them to the appropriate Office, where they will be maintained for an extended period of time in a manner that will ensure their ready access as needed by the NCI.

IV.C. Personnel Practices

IV.C.1. Protective Clothing

IV.C.1.a. Laboratory Personnel Protective clothing, such as a fully fastened laboratory coat, shall be worn in any work area where chemical carcinogens are being used. Gloves that are appropriate to the specific situation shall be used when handling chemical carcinogens. Clean clothing shall be provided daily and shall not be worn outside the work area once the work area has been entered. Clothing contaminated by chemical carcinogens shall be decontaminated or disposed of immediately after an overt exposure. Clothing contaminated with chemical carcinogens shall not be sent out for laundering until decontaminated.

IV.C.1.b. Animal Care Personnel Animal care and other personnel entering an area where animals are being treated with chemical carcinogens shall use a complete clothing change, including pants and shirts or jumpsuits, shoes or boots, head cover, and gloves. Clean clothing shall be provided daily and shall not be worn outside the work area once it has been entered. Clothing contaminated by chemical carcinogens shall be decontaminated or disposed of immediately after an overt exposure.

IV.C.2. Protective Equipment

Personnel engaged in animal or other procedures where exposure to airborne particulates contaminated with chemical carcinogens could occur shall wear an appropriate face mask or respirator. The selection of an appropriate face mask or respirator shall be made according to the guidance of the Office of Research Safety. The face mask or respirator shall not be worn outside the work area. Used filters shall be disposed of and the respirator housing shall be decontaminated daily.

IV.C.3. Showers

Personnel engaged in animal procedures or any other procedure where exposure to airborne particulates contaminated with chemical carcinogens could occur shall shower after each exit from the work area. All personnel shall shower immediately after any overt exposure to a chemical carcinogen.

IV.C.4. Eating, Drinking, and Smoking

There shall be no eating, drinking, smoking, chewing of gum or tobacco, application of cosmetics, or storage of food in areas where chemical carcinogens are used.

IV.C.5. Pipetting

Mechanical pipetting aids shall be used for all pipetting procedures. Oral pipetting shall be prohibited.

IV.C.6. Personal Hygiene

All personnel shall wash their hands immediately after completion of any procedures in which chemical carcinogens have been used.

IV.D. Operational Practices

IV.D.1. Work Area Identification

Entrances to all work and storage areas where chemical carcinogens are present shall be posted with signs bearing the legend:

DANGER--CHEMICAL CARCINOGEN
Authorized Personnel Only

IV.D.2. Access Control

Work and storage areas where chemical carcinogens are present shall be entered only by personnel authorized by the Laboratory Supervisor. Access procedures shall be prominently displayed at points of access.

IV.D.3. Work Surfaces

All work surfaces (bench tops, hood floors, etc.) on which chemical carcinogens are used shall be covered with stainless steel or plastic trays, uncracked glass plates, dry absorbent plastic-backed paper, or other impervious material. The protective surfaces shall be examined for possible contamination immediately after the procedure involving the chemical carcinogen has been completed. The contaminated surface shall be decontaminated or disposed of as is appropriate.

IV.D.4. Use of Laboratory-Type Hoods

Procedures that involve the use of chemical carcinogens shall be conducted in a laboratory-type hood or other suitable containment device when: (1) the procedure involves the use of volatile chemical carcinogens; or (2) the procedure results in the generation of aerosols, such as from the opening of closed vessels, transfer operations, weighing, preparation of feed mixtures, and the application, injection and intubation of a chemical carcinogen to experimental animals. Each laboratory-type hood or containment device used for containment of chemical carcinogens shall display a label bearing the legend:

DANGER--CHEMICAL CARCINOGEN

IV.D.5. Working Quantities

Only minimum working quantities of chemical carcinogens shall be present in a work area.

IV.D.6. Identification, Storage, and Inventory

IV.D.6.a. Labeling Storage vessels containing chemical carcinogens shall be labeled:

DANGER--CHEMICAL CARCINOGEN

IV.D.6.b. Storage Stock quantities of chemical carcinogens shall be catalogued and stored in a specific storage area that is secured at all times.

IV.D.6.c. Inventory An inventory of all chemical carcinogens shall be maintained by the Laboratory Supervisor. The inventory records shall include the quantities of chemical carcinogens acquired, dates of acquisition, and disposition. A copy of the inventory records shall be furnished to the Office of Research Safety on a semi-annual basis.

IV.D.7. Laboratory Transport

IV.D.7.a. Stock Quantities of Chemical Carcinogens An unbreakable outer container shall be used to transport chemical carcinogens.

IV.D.7.b. Contaminated Materials Materials contaminated with chemical carcinogens that are transferred from work areas to disposal areas shall be placed into separate closed, plastic bags, or other suitable impermeable, and sealed containers for each carcinogen and labeled with both the name of the carcinogen and "DANGER--CHEMICAL CARCINOGEN," before being transported.

IV.D.8. Houskeeping

General housekeeping procedures which suppress the formation of aerosols, such as the use of a wet mop or a vacuum cleaner equipped with a HEPA filter on the exhaust, shall be used. Dry sweeping and dry mopping are prohibited because of the hazard of aerosol formation. In those instances where a chemical-containing material is spilled, special procedures shall be followed.

IV.D.9. Protection of Vacuum Lines

Each vacuum service shall be protected with a disposable HEPA filter and liquid trap to prevent entry of any chemical carcinogen into the vacuum system. When using a volatile carcinogen a separate vacuum pump or other device shall be used in conjunction with an appropirate laboratory-type hood or other containment device approved by the Office of Research Safety.

IV.D.10. Packaging and Shipping

The packaging and shipping methods established by the Department of Health, Education and Welfare for the transportation of etiologic agents (42 CFR 72.25, 1972) shall be applied to the shipment of all stable chemical carcinogens. For chemical carcinogens which are physically or chemically unstable, e.g., corrosive, explosive, or flammable, the procedures established by the Department of Transportation for the handling of such materials (49 CFR 173, 1973) shall be employed.

IV.D.11. Decontamination and Disposal

IV.D.11.a. Decontamination Contaminated materials shall be decontaminated by procedures that either inactivate the chemical carcinogens or remove them for subsequent disposal.

IV.D.11.b. Disposal Contaminated wastes, cleaning devices, and animal carcasses shall be collected in impermeable containers, which are closed prior to removal from the work areas, and disposed of by appropriate methods as approved by the Office of Research Safety.

IV.D.11.c. Spills Chemical carcinogens which have spilled out of a primary container so as to constitute a hazard shall be activated *in situ* or shall be absorbed by appropriate means for subsequent disposal.

IV.D.12. Animal Housing

Animals shall be housed in a cage that confines feed, feces, urine, and bedding within the enclosure. For non-volatile carcinogens, use of a cage with solid sides and bottom in conjunction with a filter top is recommended. When using a volatile chemical carcinogen the cage must be used in conjunction with appropriate ventilation systems. Alternative animal housing methods shall require approval by the Office of Research Safety.

IV.E. Laboratory-Type Hoods and Exhaust Air-Treatment

IV.E.1. Laboratory-Type Hoods

IV.E.1.a. Open Face Hoods Open face hoods, such as a chemical fume hood, shall have an average linear face velocity of 100 feet per minute. The minimum air velocity at any point in the face of the fully opened hood shall not be less than 85 feet per minute.

IV.E.1.b. Glove Boxes Glove boxes shall be kept under negative air pressure of 0.5 inches water gauge with respect to the space in which they are located.

IV.E.1.c. Laminar Flow Biological Safety Cabinet Laminar flow biological safety cabinets may be used for the containment of *in vitro* procedures involving the use of chemical carcinogens provided that: (1) the exhaust air flow is sufficient to provide an inward air flow at the face opening of the cabinet equal to 100 feet per minute times the face opening area, (2) contaminated air plenums that are under positive air pressure are leak tight, and (3) the cabinet exhaust air is discharged outdoors.

IV.E.2. Exhaust Air Treatment

The exhaust air from laboratory type hoods and other ventilated containment devices shall be appropriately treated such as by filtration, reaction, absorption, adsorption, incineration, or dilution, so that the concentration of any chemical carcinogen or combination of chemical carcinogens in the final effluent which is discharged outdoors shall not exceed 1 ppb or natural background levels, whichever is greater. Exhaust air treatment systems that remove chemical carcinogens from the exhaust air by collection mechanisms such as filtration, absorption, and adsorption shall be operated in a manner that permits maintenance so as to avoid direct contact with the collection medium.

IV.E.3. Performance Certification

Performance of laboratory-type hoods and exhaust air treatment systems shall be certified at least annually.

IV.F. Facilities

IV.F.1. General Ventilation Control

IV.F.1.a. General Exhaust Air The general exhaust air from work areas in which chemical carcinogens are used shall be discharged outdoors and dispersed to the atmosphere so as to prevent re-entry into the facility. No recirculation of exhaust air from work areas is permitted.

IV.F.1.b. Air Pressure Work areas in which chemical carcinogens are used shall be kept under negative air pressure with respect to the access corridor. For facilities where work areas have "clean" access corridors and "dirty" egress corridors, the "dirty" egress corridor shall be kept under negative air pressure with respect to the work area.

IV.F.2. Change Rooms and Showers

Clothing change rooms and showers shall be available to personnel engaged in activities involving chemical carcinogens.

V. GLOSSARY

Given below are some definitions of terms used in the *NCI Safety Standards for Research Involving Chemical Carcinogens*.

ACCIDENT -- is an unforseen circumstance or set of circumstances resulting in the release of a chemical carcinogen that may result in contact with or exposure to a chemical carcinogen.

AUTHORIZED PERSONNEL -- are those employees whose duties require them to be in work or storage areas where chemical carcinogens are present, and who are specifically assigned there by the Laboratory Supervisor.

CHEMICAL CARCINOGEN -- is a chemical that has been demonstrated to cause tumors in mammalian species by induction of a tumor type not usually observed; or

by induction of an increased incidence of a tumor type normally seen, or by its appearance at a time earlier than would be otherwise expected.

DECONTAMINATION -- is the safe removal of a chemical carcinogen from a contaminated item.

DISPOSAL -- is the safe elimination of a chemical carcinogen from the general environment by inactivation, degradation, destruction, or other appropriate method.

EMERGENCY -- is an accident or other event that requires immediate action.

GLOVES -- are covers to protect the hands of a worker against contact with or exposure to a chemical carcinogen.

GLOVE BOX -- is a fully enclosed ventilated containment device, provided with port-holes to which are attached flexible gloves that permit the insertion of hands within the device, to perform manual operations without contact with or exposure to a chemical carcinogen.

HEPA FILTER -- is a High Efficiency Particulate Air Filter that is capable of retaining 99.97% of a mono dispersed aerosol of 0.3 m particles.

LABORATORY SUPERVISOR -- is a person who plans and directs research projects and other activities in the laboratory.

LABORATORY WORKER -- is a person who carries out an assigned task in the laboratory.

LAMINAR FLOW BIOLOGICAL SAFETY CABINET -- is a ventilated containment device that provides both personnel protection and a contamination-free work environment. Personnel protection is achieved by an inflow of room air at the work opening where it is quickly entrained in a recirculating air stream and removed through an exhaust

grille at the leading edge of the work area. A contamination-free work zone is achieved by supplying air through High Efficiency Particulate Air Filters downward towards the work surface at a uniform velocity. Air flow equal to the in flow of room air is exhausted through High Efficiency Particulate Air Filters incorporated in the cabinet or the exhaust system.

MONITORING -- is to determine, on a regular basis, the possible presence of a specific chemical carcinogen in the environment by means of a sensitive analytical method.

OPEN FACE LABORATORY TYPE HOOD -- is a device enclosed on three sides and the top and bottom; is designed and maintained to draw air inward at an average linear face velocity of 100 feet per minute; and is designed, constructed, and maintained so that an operation involving a chemical carcinogen within the hood does not require the insertion of any portion of a worker's body other than his hands and arms.

PROTECTIVE CLOTHING -- are clothes designed to protect a worker against contact with or exposure to a chemical carcinogen.

PROTECTIVE EQUIPMENT -- is equipment in addition to protective clothing and gloves, such as a face mask or a respirator, that is designed to protect a worker against contact with or exposure to a chemical carcinogen.

SAFETY ACTION PLAN -- is a plan that describes the procedures and mechanisms, including operational practices, emergency procedures, and monitoring techniques, to be used in implementing the NCI Safety Standards for Research involving Chemical Carcinogens.

STORAGE AREA -- is the area where a chemical carcinogen not required for daily usage is stored.

WORK AREA -- is an area where entry and exit is restricted and controlled because of the presence of a chemical carcinogen.

WORKING QUANTITY -- is that amount of chemical carcinogen required for carrying out a project on a day-to-day basis.

APPENDIX D

ANIMAL RANDOMIZATION

Randomization of the animals is necessary to ensure that unintentional selection biases are not introduced into the bioassay study. All the animals should be from the same supply source and, ideally, within 2-3 days of the same age. Unsuitable animals should be culled and discarded during the quarantine period (see Section III.C.). Optimally, animals should be assigned to experimental groups and cages by the use of a table of random numbers. In this instance, each animal must be consecutively numbered. An example of such a randomization procedure is shown below.

Random assignment is to be made of 150 animals to three experimental groups so that there are 50 in each group. The animals are consecutively numbered. A starting place in a table of random numbers is selected and successive triplets of numbers listed, omitting 000, numbers greater than 150, and repeats. The first 50 numbers correspond to animals assigned to group one, the second 50 to group two, and the remaining 50 to the third group. When animals are segregated into weight distribution groups prior to randomization, this procedure is repeated completely for each of these groups. This will ensure that there will be an equal number of animals from each weight distribution group in each of the experimental groups.

APPENDIX E

BIOASSAY PATHOLOGY PROCEDURES

These *Bioassay Pathology Procedures* have been prepared by Dr. Robert A. Squire, Head, Tumor Pathology Section, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute.

The purpose of this document is to assure uniform and scientifically acceptable pathologic procedures in the NCI Carcinogenesis Bioassay Program. It is to be implemented in all studies in which the effects of possible carcinogens may be systemic, regardless of the route of administration. The procedures are not necessarily intended for specific target-tissue carcinogenesis studies or for other toxicologic evaluations.

I. Personnel

A. A board-certified veterinary or medical pathologist with experience in laboratory animal pathology shall be responsible for all pathology procedures, evaluations, and reporting. If not board-certified, persons may be acceptable if appropriate training and experience judged to be satisfactory by NCI can be demonstrated.

B. Histology technician(s) shall be supervised by an HT/ASCP registered technician, judged to be qualified by NCI. If not certified, persons may be acceptable if appropriate training and experience judged to be satisfactory by NCI can be demonstrated.

C. Prosectors shall be trained and experienced in laboratory animal dissection and able to recognize and describe gross abnormalities. Careful performance of the necropsy for the detection of possible tumors at any site is vital to carcinogenesis experiments. Therefore, qualified and well-supervised personnel are necessary.

D. Personnel must be available for weekend coverage to necropsy dead or moribund animals.

II. Facilities

A. Refrigeration shall be available for holding dead animals until necropsy. Animals should not be frozen.

B. The gross necropsy facility should be in close proximity to the pathologist's office(s) and equipped with adequate working surfaces, dissection boards, running water with drains, adequate lighting, ventilation, exhaust hoods and gross photography capabilities.

C. The histology laboratory shall be separate from the necropsy area and equipped with automatic tissue processor, microtomes, embedding and staining equipment, and supplies.

D. Adequate storage facilities are required to store and file histologic slides, tissue blocks, and wet tissues for duration of the contract. This facility should be vermin proof and temperature controlled.

E. An area for trimming of fixed tissues, with adequate ventilation and exhaust hoods, is required.

III. Gross Necropsy

A. All animals which die or are sacrificed in the repeated dose, subchronic, and chronic studies shall be necropsied (unless cannabilism or autolysis precludes the examination).

B. The gross dissection and evaluation shall be performed by or under the direct supervision of the pathologist(s).

C. The necropsy is defined as external examination, including body orifices, and examination and fixation of all of the following tissues:

Gross lesions	Lungs & bronchi
Tissue masses or suspect tumors & regional lymph nodes	Heart
	Thyroids
Skin	Parathyroids
Mandibular lymph node	Esophagus
Mammary gland	Stomach
Salivary gland	Duodenum
Larynx	Jejunum
Trachea	Ileum

Cecum	Spleen
Colon	Kidneys
Rectum	Adrenals
Mesenteric lymph node	Bladder
Liver	Seminal vesicles
Thigh muscle	Prostate
Sciatic nerve	Testes
Sternebrae, vertebrae, or femur (plus marrow)	Ovaries
	Uterus
Costochondral junction, rib	Nasal cavity
	Brain
Thymus	Pituitary
Gallbladder	Eyes
Pancreas	Spinal cord

D. Specific Methods

1. Peripheral blood smears shall be prepared from all animals at sacrifice, immediately air-dried, and fixed in absolute methanol within 24 hours.

2. All tissues and/or organs are to be examined *in situ*, then dissected from the carcass, reexamined, including cut surfaces, and fixed in 10% neutral buffered formalin.

3. Lungs of mice and rats may be fixed in their entirety after opening and examining the trachea and mainstem bronchi.

4. The calvarium shall be removed and the dorsal nasal bone removed for examination of nasal turbinates. The entire skull may be fixed with the brain *in situ*.

5. Other tissues shall be fixed at a thickness not exceeding 0.5 cm.

6. Distended urinary bladders shall be opened and examined before fixation. Contracted, empty bladders shall be partially distended with formalin and opened and examined after fixation.

7. Liver lobes shall be sliced, the kidneys longitudinally bissected, and the cut surfaces examined before fixation.

8. The entire mucosal surfaces of the esophagus, stomach, small and large intestines, and rectum shall be opened and examined before fixation.

9. Multiple, representative portions of large or variable tissue masses including surrounding unaffected tissues shall be fixed.

10. Several thoraco-lumbar vertebrae shall be fixed with the spinal cord *in situ*.

E. All gross lesions shall be recorded in narrative, descriptive terms including location, size (in mm), number, shape, color, and texture.

F. Carcasses of animals may be discarded immediately following necropsy and fixation of all tissues listed under III C.

IV. Fixed Tissue Trimming

A. It is desirable that fixation time be no less than 48 hours nor more than 12 weeks.

B. Tissue trimming shall be performed by or under the supervision of the pathologist(s) with the gross necropsy descriptions available. Additional gross observations shall be recorded at the time of trimming if necessary.

C. Tissues shall be trimmed to a maximum thickness of 0.3 cm for processing.

D. Specific methods

1. Multiple portions of tumors or masses shall be submitted if these are large or variable in appearance. Surrounding normal tissue shall be included.

2. Parenchymal organs, e.g., liver, shall be trimmed to allow the largest surface area possible for examination.

3. Mid-longitudinal sections through the entire cortex and medulla of each kidney shall be submitted.

4. Entire coronal (a transverse section parallel to the long axis of the body) sections of both right and left lungs including main-stem bronchi shall be submitted.

5. Three cross sections of brain shall include: (a) frontal cortex and basal ganglia, (b) parietal cortex and thalamus, and (c) cerebellum and pons.

6. Hollow organs shall be trimmed and blocked to allow a cross-section slide from mucosa to serosa.

V. Histologic Technique

A. Tissues shall be cut at 4-6 μ and stained with hematoxylin and eosin (H & E).

B. Blood smears shall be air-dried at necropsy, then fixed in absolute methanol for five minutes. Smears from animals with anemia, enlarged thymus, lymphadenopathy, or hepatosplenomegaly shall be stained with Wrights, Giemsa, or similar Romanovsky stain.

VI. Histopathologic Examinations

A. Animals to be subjected to histopathologic examination:

1. All control animals (see * on page 56), highest dose-level animals without mortality, and the next higher dose-level group in subchronic studies.

2. All control (see * on page 56) and treated animals in chronic studies.

B. Histopathologic examination is defined as histologic examination of the following:

gross lesions
tissue masses or suspect tumors & regional lymph node(s)
blood smear (if anemia, enlarged thymus, lymphadenopathy or hepatosplenomegaly, is present)
mandibular lymph node
mammary gland
salivary gland
sternebrae, femur or vertebrae including marrow
thymus
trachea
lungs & mainstem bronchi
heart
thyroids
parathyroids
esophagus
stomach
small intestine (one section)
colon
liver
gallbladder
pancreas
spleen
kidneys
adrenals
bladder
prostate
testes
ovaries
uterus
brain (three sections including frontal cortex & basal ganglia, parietal cortex & thalamus, & cerebellum & pons)
pituitary
eyes (if grossly abnormal)
spinal cord (if neurologic signs are present)

VII. Pathology Reports (see Individual Animal Data Record, p. 28)

A. Descriptive narratives of gross necropsy findings are required for all animals. The number as well as description of tissue masses should be included. If these are confluent or too numerous to count (TNTC), this should be indicated.

B. One narrative histologic description per animal test group of each different histopathologic diagnosis is required. This may be done the first time a given lesion (including tumors of the same type) is observed in a group.

C. Histopathologic diagnoses of all lesions shall be entered under *Organ and Diagnosis*. Indicate primary versus metastatic tumors, e.g.:

1. Liver, hepatocellular carcinoma

2. Lung, hepatocellular carcinoma, metastatic

3. Kidney, interstitial nephritis, chronic

VIII. To be Retained until Termination of Study and Final Report

A. All wet tissues, preferably plastic-bagged, shall be clearly and permanently labeled and retained in a vermin-proof area.

B. All histologic slides and paraffin blocks shall be retained under suitable temperatures.

C. No material shall be discarded without prior permission of NCI.

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* Positive control animals shall be subject to complete gross necropsy and tissues fixed as in III C. Tissues to be subjected to histopathologic examination will be determined by consultation with the NCI project officer and will be sufficient to determine tumor types and incidence.

IX. Pathology Material to be Submitted to the NCI

A. One H & E stained slide plus the tissue block representative of each different neoplasm or treatment-related lesion from each chemical test group shall be sent to the Tumor Pathology Section when the test is completed or sooner, if possible, or if consultation is desired.

B. All pathologic specimens are the property of NCI and shall be submitted to NCI upon request.

APPENDIX F

CARCINOGEN BIOASSAY INFORMATION

For each bioassay study done on a particular chemical, the information listed below should be collected. This information conforms with the recommendations made in the report by the *Panel on Carcinogenicity Testing of the International Union Against Cancer* (Berenblum, 1969).

GENERAL:

1. Outline of the bioassay study
2. Bioassay study number in the investigator's file
3. Names of the investigators responsible for the bioassay study, including histopathological diagnoses
4. Name of bioassay laboratory

CHEMICAL:

1. Name, Chemical abstract number, NCI number
2. Name, synonyms
3. Formula
4. Source (generic)
5. Manufacturer
6. Batch number
7. Date(s) when received
8. Storage (before its reception)
9. Physical state and other characteristics
10. Melting point

11. Solubility
12. Criteria of purity
13. Impurities (generic)
14. Methods of synthesis
15. Storage conditions and dates
16. Other

PREPARATION:

1. Chemical(s): name, data sheet number
2. Vehicle(s): name, data sheet number
3. Preparation and concentrations
4. Methods of preparation
5. Amount prepared each time
6. Frequency of preparation
7. Physical state
8. pH
9. Stability and decomposition
10. Storage
11. Date(s) prepared
12. Other

ANIMALS:

1. Species
2. Strain and subline
3. Initial number by sex (male and female)
4. Date(s) of birth (male and female)
5. Source
   a. Own colony (give reference)
   b. Other

6. Breeding
   a. Inbred
   b. Random
   c. Outbred
   d. Other

7. Disease Control
   a. Specific pathogen-free
   b. Germ-free
   c. Conventional
   d. Vaccinated

8. Distribution in groups
   a. Pooled at weaning
   b. Random
   c. Random tables
   d. Littermates
   e. Other

9. Other experimental groups included in the same distribution

10. Initial number per cage

11. Divided by sex

12. Age when obtained from Animal House

13. Maintenance (general conditions)
   a. Own standard (give reference)
   b. Special

14. Cages

15. Bedding

16. Room temperature (range)

17. Light
   a. Source
   b. Time cycle

18. Diet
   a. Type
   b. Source

19. Amount of diet
   a. *Ad libitum*
   b. Measured

20. Water
    a. Tap
    b. Other

21. Amount of water
    a. *Ad libitum*
    b. Measured

22. Other or special conditions

TREATMENT:

1. Special pretreatment conditions

2. Treatment multiplicity
    a. Single type
    b. Combined

Item 3 - 14 should apply for each treatment

3. Preparation administered

4. Dose per administration
    a. Volume
    b. Weight
    c. How measured

5. Route

6. Site

7. Methods and instruments used
    a. Surgical procedures
    b. Anesthesia
    c. Sterility

8. Frequency of administration

9. Total number of doses

10. Total dose given

11. Total time of treatment

12. Date treatment started

13. Date treatment ended

14. Others

PLAN OF OBSERVATIONS:

1. Age of animals at start of experiment
2. Weight of animals at start of experiment
3. Duration of experiment
   a. Lifespan
   b. Interruptions (from when to when and why)
4. Frequency of checking
5. Frequency of weighing
6. Frequency of charting
7. Frequency of measuring consumption of:
   a. Feed
   b. Water
8. Other observations
9. Autopsies
   a. On all animals
   b. With the exception of:
      (1) decomposed animals
      (2) lost animals
      (3) other
10. Autopsy examinations
    a. Complete
    b. Except cranial cavity
    c. Other exceptions
11. Histology
    a. All tumors (note exceptions)
    b. Other tissues
12. Other pathological observations

CONTROLS:

1. List:
   a. Each group
   b. Selection

VARIATIONS:

1. List protocol additions or changes

REPORTS:

1. Animal groups
   a. Body weight curves

2. Individual animals
   a. Identification number
   b. Mode of death (died or sacrificed)
   c. Time of death (in days or weeks of age, or time from start of bioassay study)
   d. Diagnosis of tumors found at necropsy and other pertinent pathology
   e. Indication if necropsy not done (as in decomposition) and animal is considered lost from the study

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