

NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS

December 14 and 15, 1987

Summary Minutes

National Toxicology Program
Board of Scientific Counselors Meeting

December 14 and 15, 1987
Summary Minutes

<u>Contents</u>	<u>Page Numbers</u>
<u>Review of NIEHS Systemic Toxicology Branch Programs in Immunotoxicology and Chemical Disposition</u>	
I. <u>Overview</u>	1
II. <u>Immunotoxicology</u>	
A. <u>Program Overview</u>	1
B. <u>Contract Overview</u>	2
C. <u>Overview of Lung Immunotoxicology Program</u>	2
D. <u>Examination of PMN Function in Immunotoxicology</u>	2
E. <u>Studies in B Lymphocyte Maturation Using Chemical Probes</u>	2
F. <u>Future Directions</u>	2
III. <u>Chemical Disposition</u>	
A. <u>Introduction</u>	3
B. <u>Metabolism, Ageing, and Toxicity</u>	3
C. <u>Xenobiotic Metabolism</u>	3
D. <u>Butoxyethanol Induced Hematotoxicity</u>	4
E. <u>Mechanisms of Forestomach Toxicity</u>	4
F. <u>Chemical Disposition Contracts and Interagency Agreement</u>	4
1. <u>Chemical Disposition</u>	4
2. <u>Inhalation Studies</u>	5
3. <u>Human Metabolism Studies</u>	5
G. <u>Conclusions and Future Plans</u>	5
IV. <u>Report of the Director, NTP</u>	5
V. <u>NIEHS/NTP Concept Review - "Development of Mutagenesis Assays Using Transgenic Mice"</u>	6
VI. <u>Patterns of Growth, Survival and Tumor Trends in Rats and Mice from 1971-1983</u>	6
VII. <u>NIEHS Dietary Restriction Studies in Rodents</u>	7
VIII. <u>NCTR Caloric Restriction Studies in Rodents</u>	7
IX. <u>Review of Chemicals Nominated for NTP Studies</u>	8

Attachments 1-5

NATIONAL TOXICOLOGY PROGRAM
BOARD OF SCIENTIFIC COUNSELORS MEETING
December 14 and 15, 1987

SUMMARY MINUTES

The National Toxicology Program (NTP) Board of Scientific Counselors met on December 14 and 15, 1987, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, North Carolina. (Attachment 1: Federal Register Meeting Announcement; Attachment 2: Agenda and Roster of Members and Expert Consultants.) Members of the Board are Drs. Michael Gallo (Chairman), Richard Griesemer, John Little, Frederica Perera, Adrienne Rogers, Robert Scala, and Arthur Upton.

Review of NIEHS Systemic Toxicology Branch Programs in Immunotoxicology and Chemical Disposition

I. Overview: Dr. Bernard Schwetz, Chief, Systemic Toxicology Branch (STB), Division of Toxicology Research and Testing (DTRT), NIEHS, gave a brief overview of the STB noting there were five workgroups. Besides the workgroups to be reviewed, there are also workgroups in developmental and reproductive toxicology, inhalation toxicology and metals toxicology. The Branch objectives are in applied research to characterize the toxicology of chemicals and to develop, evaluate and validate appropriate methodology. He concluded by outlining how the programs to be reviewed fit into the overall toxicological evaluation process of the NTP.

The review format used for both immunotoxicology and chemical disposition programs combined platform presentations with poster sessions which allowed for more informal and indepth interactions among reviewers and program staff. The posters were displayed in the lobby areas adjacent to the conference room.

II. Immunotoxicology:

A. Program Overview - Dr. Michael Luster, Head, began with a schematic and functional description of the immune system. He pointed out the diverse types of chemicals and chemical classes that have been associated with immunological abnormalities, in a number of cases in both rodents and humans. The inhouse program is divided into: (1) methods development and validation; (2) chemical and drug evaluation; and (3) mechanistic studies.

Methods development activities include development and application of an assay battery, developing means to assess polymorphonuclear neutrophil (PMN) activity and functions, and developing and validating methods to measure pulmonary alveolar macrophage capacity and functions. Among studies evaluating chemical and drug effects are those examining: the relationship between immune function disturbance and hepatocellular carcinogenesis; the effects of ochratoxin on natural killer (NK) cell function and interferon levels; the immunotoxicity of antifolates; and the immunotoxicity of experimental therapeutic agents for AIDS. Among mechanistic studies is the development of B cell maturation as an in vitro model to determine the biochemical and cellular events responsible for immuno-suppression by chemicals.

B. Contract Review - Dr. Luster noted there was one ongoing contract divided into three tasks. Task I was to establish an immune testing panel. The current panel is composed of Tier I (Screen) and Tier II (Comprehensive) with both tiers incorporating measures of immunopathology and alterations in humoral-mediated, cell-mediated, and non-specific immunity while Tier II also includes host resistance challenge models. Agents exhibiting a positive response(s) in Tier I may then be evaluated in Tier II. Under Task II (ongoing), four chemicals/year are evaluated for their potential to induce immune alterations (either suppression or potentiation) while four/year are evaluated for their ability to induce contact hypersensitivity. Task III represents an in-depth examination of cellular and subcellular events associated with chemical-induced immunotoxicity for one chemical yearly. Dr. Luster illustrated the process using gallium arsenide.

C. Overview of Lung Immunotoxicology Program - Dr. Gary Rosenthal discussed immune responses in the respiratory tract noting that the lung is often the first line of defense against xenobiotics and an important component of this defense is the pulmonary alveolar macrophage (PAM). Discussed were the suppressor versus stimulatory actions of xenobiotics on PAM function. Dr. Rosenthal said he was in the process of developing, characterizing, and validating a battery of endpoints with which to examine PAMs in vitro, as well as following in vivo exposure to specific chemicals. He reported on various markers for PAM activation including Ia antigen expression, H_2O_2 production, tumor necrosis factor (TNF) secretion - especially in response to stimulation by inhaled particulates, and the apparent role of these PAM secretory products as mediators in the development of pulmonary fibrosis.

D. Examination of PMN Function in Immunotoxicology - Dr. Michael Ackermann said they were concerned with the need and relevance of polymorphonuclear neutrophils (PMN) and the effects of xenobiotic exposure on PMN function. An assay panel was developed to study xenobiotic effects. Among assays developed was a method for studying the cytolytic/cytostatic activity of PMN, using a flow cytometry technique.

Dr. Ackermann described studies on the effects of 2, 3, 7, 8-tetrachloro-dibenzo-p-dioxin (TCDD) on various aspects of PMN activity. He focused on the inhibition by TCDD of the cytolytic/cytostatic activities of PMNs. The reduction in these actions caused by TCDD could not be explained by decreases in superoxide production or hydrogen peroxide release but rather by a reduction in the release of cytolytic factor by PMNs.

E. Studies in B Lymphocyte Maturation Using Chemical Probes - Dr. Luster stated that the majority of xenobiotics that cause immunosuppression do so by affecting antibody (B cell) responses. There are good in vitro models for studying the three stages of B cell maturation, i.e., activation, proliferation, and differentiation. He listed the assays employed to monitor the process. Dr. James Blank described in detail studies on the immunological and biochemical effects of pertussis toxin on B lymphocyte maturation as a means of understanding its mechanism of toxicity. Dr. Luster then reported on studies which have dissected the characteristics of TCDD immunotoxicity. Studies with congenic mice have shown immunosuppression occurs only in Ah responsive mice. TCDD acts to prevent differentiation with little effect on the earlier stages of B cell maturation.

F. Future Directions - Dr. Luster said the programs would continue to evaluate potentially immunotoxic chemicals and attempt to elucidate mechanisms

responsible, primarily in humans. Emphasis in methods development and validation will be placed on development of in vitro models. Among new initiatives are: (1) collaborative studies on the comparative immunotoxic and myelotoxic effects of some of the more promising antiviral agents against AIDS; (2) continued development of pulmonary immunology models to examine responses following inhalation exposure to various chemicals. Cells to be evaluated include natural killer (NK) cells and lymphocytes while chemicals include arsine and methylene chloride; (3) collaborative studies to examine immunotoxic effects of selected chemicals on freshly isolated human lymphocytes; and (4) development of a T cell activation model.

III. Chemical Disposition:

A. Introduction - Dr. H. B. Matthews, Head, stated the objectives of studies in chemical disposition to be - short-term: (1) support of NTP study design; (2) provide additional toxicological characterization for selected chemicals; and (3) help explain results of toxicology and carcinogenesis studies; - long-term: (1) to explore chemical structure-activity relationships (SAR); (2) determine mechanisms of toxicity; and (3) develop better information for extrapolation of animal data to humans. The group is involved both prospectively through involvement in Program chemical selection and during the design of protocols for in vivo studies but also retrospectively to conduct studies aimed at explaining toxicity observed or sex/species differences in toxicity. About 16 chemicals are studied yearly, generally in adult male F344 rats. Dr. Matthews reviewed the chemicals and chemical classes for which chemical disposition studies have been or are being conducted. He described the resources allocated to the program, the contracts in force and time commitments by the staff. Disposition studies done in his own laboratory since 1984 (the last program review) included methyl and ethyl carbamates, resorcinol, 1,2-dihydro-2,2,4-trimethylquinoline, butyl acrylate, and dimethyl hydrogen phosphite. Dr. Matthews used the results on methylcarbamate to illustrate how species differences in toxicity could be evaluated through chemical disposition studies.

B. Metabolism, Ageing, and Toxicity - Dr. Linda Birnbaum discussed some of the disposition studies conducted in her laboratory since the last Board review in 1984: (1) maintenance of a colony of congenic mice to evaluate the influence of the Ah locus, e.g., disposition of TCDD is independent of the locus but dependent on degree of body fat; (2) o-benzyl-p-chlorophenol is more toxic dermally than orally, and, may be a tumor promotor in skin; (3) demonstration of dramatic differences in rates of disposition for positional isomers of polyhalogenated aromatics; (4) pursued the use of in situ luminal perfusion techniques in oral absorption studies; (5) studied effects of ageing (senescence) on gastrointestinal absorption of chemicals - no effect on chemicals passively absorbed versus decreased absorption for chemicals requiring some type of active transport; (6) evaluated effects of ageing on metabolism and toxicity of chemicals; (7) toxicity studies with perfluorodecanoic acid and octa-chlorodibenzo-p-dioxin; (8) studies of the relative teratogenicity of various dioxins and furans in the developing mouse embryo, and on the mechanisms of cleft palate formation in rats; and (9) studies on the interactions of TCDD and congeners with various growth factors and hormone receptors in different tissues.

C. Xenobiotic Metabolism - Dr. L. T. Burka emphasized efforts on the isolation and identification of xenobiotic metabolites, enzymatic mechanisms especially for the mixed function oxidases, and interactions of xenobiotics and

their metabolites with tissue macromolecules. Dr. Burka described the biotransformation of chemicals studied recently, including: 1,2-dihydro-2,2,4-trimethylquinoline; 5-(4-nitrophenyl)-2,4-pentadienal (Spydust); butyl and ethyl acrylates; 2-butoxyethanol; citral; methallyl chloride; dimethylvinyl chloride; and furan. Metabolites were usually isolated by high performance liquid chromatography and metabolite structures are established by nuclear magnetic resonance and/or mass spectrometry.

D. Butoxyethanol Induced Hematotoxicity - Dr. Burhan Ghanayem said 2-butoxyethanol (BE) in rats caused acute hemolytic anemia resulting in severe hemoglobinuria plus liver and kidney damage as a result of hemoglobin accumulation. Toxicity was shown to be caused by the major metabolite, 2-butoxyacetic acid (BAA), and was specific to this chemical structure. Older animals were more sensitive than younger ones to BE toxicity while in vitro incubations indicated that human red blood cells were less sensitive than rat red cells to the hemolytic effects of BE.

E. Mechanisms of Forestomach Toxicity - Dr. Ghanayem noted that a number of chemicals in long-term studies have been associated with neoplasms in the forestomach of rats and mice when given by gavage. Ethyl acrylate (EA) was chosen as a model chemical to study this lesion. His studies showed rapid absorption and metabolism, binding to protein, and increased cell proliferation, an effect identified as being characteristic of chemicals which cause forestomach tumors.

F. Chemical Disposition Contracts and Interagency Agreement - Dr. Matthews noted that collaborative projects also are reviewed by ad hoc committees.

1. Chemical Disposition - there are three ongoing contracts and one which expired in 1986. He described some of the studies done under each contract:

a) Southern Research Institute: studies with sunscreen agents, e.g., 1-hydroxy-4-methoxybenzophenone (HMB), showed appreciable dermal absorption but no evidence of toxicity or persistence of HMB or metabolites; and studies with N-phenyl-2-naphthylamine indicated that the carcinogen, 2-naphthylamine, was not formed as a metabolite.

b) University of Arizona: studies of the toxic interactions of eight halogenated hydrocarbons (Superfund Chemicals); and species differences in epoxide formation and hydrolysis of 4-vinylcyclohexene were consistent with tumorigenicity patterns.

c) Research Triangle Institute: studies with three glycol ether acetates showed rapid and quantitative hydrolysis in blood to parent ethers suggesting chronic studies on the acetates may not be necessary while studies with a chemical used extensively in permanent press treatment of fabric indicated normal dermal absorption.

d) Arthur D. Little, Inc.: studies with furfuryl alcohol and furfural showed their metabolism and disposition was almost identical indicating that one two-year study should be adequate to characterize the toxicity of both compounds.

2. Inhalation Studies - Dr. Birnbaum discussed the interagency agreement with the Department of Energy at the Lovelace Biomedical and Environmental Research Institute to study disposition of chemicals requiring inhalation exposure. Program interest in volatile chemicals such as benzene and 1,3-butadiene have lead to expansion of the objectives to include more extensive metabolite characterization, macromolecular interactions, and cytogenetic studies as well as a recent focus on development of physiological pharmacokinetic models, e.g., vinylidene fluoride. Among other chemicals studied were 2,3-dichloropropane, 1-chloro-2-propanol, methyl bromide, azodicarbonamide, isoprene, styrene, and three glycol ethers.

3. Human Metabolism Studies - Dr. Burka said three contracts were in place to develop methods for studying the metabolism of xenobiotics in human tissues. Sources of tissues have been identified, procedures for transporting tissues to the laboratories with minimal loss of metabolic activity have been developed, and cryopreservation techniques are being explored with a goal being the formation of tissue banks. The comparative studies of in vitro metabolism of xenobiotics in rodent and human tissues is underway. Dr. Burka described types of studies being conducted:

a) University of Arizona: developed a technique for preparing thin tissue slices and maintaining their metabolic integrity for selected substrates up to 24 hours, and have been evaluating pig liver as a potential better model than rat liver for human liver.

b) SRI International: developed techniques for isolating large numbers of viable human hepatocytes and carried out comparative metabolism studies in rat and human hepatocytes with benizidine, caffeine and trichloroethylene.

c) Mayo Clinic and Foundation: gained access to human liver and kidney samples and have maintained viable xenobiotic metabolizing enzyme activity for some substrates for up to one year. Considerable effort with mixed results has been given to developing means of cryopreservation of hepatocytes and liver slices. Vitrification as a cryopreservation technique is currently being evaluated.

G. Conclusions and Future Plans - Dr. Matthews plans to continue to design and conduct studies of chemical disposition on chemicals of interest to the NTP as well as more mechanism-oriented studies whenever it is apparent that additional data would facilitate experimental designs or interpretation of data from chronic studies. More specifically, there will be: studies on toxic mechanisms in organs such as kidney and urinary bladder analogous to studies on the forestomach; continuing studies on the effects of ageing on metabolism and toxicity; studies of receptor mediated toxicity, e.g., with dioxins; continuing studies on metabolism of xenobiotics by human tissues; and continuing development of pharmacokinetic models.

IV. Report of the Director, NTP: Dr. David Rall reported that: (1) the NIEHS should know soon what their final FY 1988 budget will be. About \$4 million are anticipated for AIDS-related research; (2) he had participated recently in NIH "Town Meetings" initiated by Dr. Wyngaarden to explain to the scientific community what is happening to the NIH budget emphasizing that in terms of real dollars the budget has increased from \$3.57 billion in 1981 to \$4.5 billion in 1988. One aim was to try and convince young scientists that there was still a

bright future in biomedical research; (3) The International Symposium on Benzene Metabolism, Toxicity and Carcinogenesis would be held at NIEHS, March 14-16, 1988, and the International Symposium on the Toxicology, Carcinogenesis and Human Health Aspects of 1,3-Butadiene would be held at NIEHS, April 12-13, 1988, both having multiple sponsors; (4) the most recent meeting of the Board's Technical Reports Review Subcommittee (Peer Review Panel) was held on November 6 at which time the Panel reviewed and approved reports of toxicology and carcinogenesis studies on benzyl alcohol, methyl dopa sesquihydrate, roxarsone, and tetracycline hydrochloride; and (5) Dr. Ernest McConnell had announced his retirement as Director, Division of Toxicology Research and Testing (DTRT), NIEHS. Dr. Rall said there would be a solicitation for candidates in Science early in January, and asked Board members to contact him with names of persons they thought would make good candidates for the position.

V. NIEHS/NTP Concept Review - "Development of Mutagenesis Assays Using Transgenic Mice:" (Attachment 3) Dr. James Mason, Project Officer, Cellular and Genetic Toxicology Branch, DTRT, noted that in vitro mutagenesis assays are limited in their ability to predict in vivo carcinogenicity as evidenced in studies at NIEHS and elsewhere that indicated about 50% of non-mutagens were carcinogenic in two-year studies while conversely there was only 70-80% positive predictivity. The objective of the concept proposal was to develop one or more in vivo mutagenesis assay systems to detect and quantitate gene mutations at a precisely defined target sequence in somatic, and possibly germ cells of mice exposed to chemicals. The development of shuttle vectors carrying mutagenesis targets and experience with transgenic mice provide the technology for engineering genetically a mutagenesis target to suit a specific need and to have that target available in every cell of the animal.

The assay system(s) developed should provide: (1) the capability to screen large numbers of chemicals in vivo; (2) the ability to analyze induced mutations at the molecular level; (3) the capability for generating a germ cell assay in the same system; and (4) the ability to compare mutagenesis results to carcinogenesis.

In discussion by the Board, it was suggested that the assays developed would constitute more sophisticated types of host mediated assays. There was enthusiasm expressed by the Board for the proposed project as allowing an examination of the mechanisms of mutagenesis and induction of the mutation process, and providing a way of understanding susceptibility at the genome level to DNA damage. Dr. Raymond Tennant, Chief, NIEHS Cellular and Genetic Toxicology Branch, commented that this could be viewed as a speculative research effort that could address issues around positive and negative mutagenicity of chemicals. He said there should be options as to approaches to be used.

Dr. Little moved that the concept be approved. Dr. Scala seconded the motion, which was approved unanimously (6 Yes votes) by the Board.

VI. Patterns of Growth, Survival and Tumor Trends in Rats and Mice from 1971-1983: (Attachment 4) Dr. G. N. Rao, Head, Laboratory Animal Management, Chemical Pathology Branch, DTRT, said that over the last 15 years observed changes in growth patterns and increases in average body weights of laboratory rats along with changes in pathology such as improved tissue accountability, better diagnostic control, and standard use of moribund sacrifice suggested there could be resulting changes in tumor trends. Dr. Rao reported on their analysis of growth, survival and tumor trends in F344 rats and B6C3F₁ mice as

obtained from the NCI/NTP data base. Tumors chosen for analysis were those for which there was a 10% or greater incidence in the historical data base. Dr. Rao discussed the data shown in Attachment 4. Time trends in rats were generally more pronounced than in mice with highly significant increases in all tumors studied as well as body weight and a corresponding decreased survival in male rats. Although improved diagnostic criteria and tissue accountability were factors, increases in average body weights were considered to be the primary factors. Since the major influence on growth and body weight is diet, Dr. Rao briefly discussed the findings and conclusions from a workshop on diet co-sponsored by the NIEHS (Attachment 4).

VII. NIEHS Dietary Restriction Studies in Rodents: Dr. Kamal Abdo, Carcinogenesis and Toxicology Evaluation Branch, DTRT, NIEHS, reported that the NTP Ad Hoc Panel on Chemical Carcinogenesis Testing and Evaluation had recommended that the NTP investigate the effect of restricted dietary feeding on tumor incidence in control animals. He reviewed the literature which shows a clear association between reduced feed intake (dietary restriction), reduced body weight, and a decrease in tumor rates in unexposed rodents especially for tumors of the mammary gland, liver, lung, hematopoietic system, pancreas, and pituitary gland. Dietary restriction has also been shown to reduce the incidence of chemically induced tumors, specifically with chemicals requiring metabolic activation. Dr. Abdo presented data on NTP studies by dosed feed and gavage routes which indicated average final body weights for high dose animals ranged from 80 to 95% of control values. In view of the high cost of dietary restriction studies and the information already available, Dr. Abdo stated that the Ad Hoc Panel's recommendations could best be met by studying the influence of dietary restriction on the carcinogenic activity of chemicals selected for study by the NTP.

In two-year studies of selected chemicals in rats, additional control groups will be added which receive a diet restricted to produce reduced body weights comparable to those seen in high dose animals fed ad libitum (ad lib). Also, high dose and control groups will receive calorically restricted, fixed (75% of ad lib control) diets. Body and organ weights, clinical chemistry indices, hematologic values, and various hormone levels will be measured. Chemicals selected for study should meet these criteria: (1) anticipated carcinogenic response is positive and the probable target organs are those likely to be influenced by restricted feeding; (2) anticipated response is negative or equivocal; and (3) the chemical is mutagenic or nonmutagenic. Based on these criteria, four chemicals were selected: t-butylhydroquinone, scopalamine hydrobromide, butyl benzyl phthalate, and salicylazo sulfapyridine.

VIII. NCTR Caloric Restriction Studies in Rodents: Dr. William T. Allaben, NCTR, reported for himself and Dr. Angelo Turturro, NCTR, who could not attend. The caloric restriction study was developed three years ago under an Interagency Agreement with the National Institute on Aging (NIA) with a major objective being to grow and age large numbers of laboratory rodents fed either ad lib or calorically restricted. These animals will be used by NIA to identify biomarkers of ageing and by NCTR to examine changes in physiologic function and to evaluate endpoints of toxicity. The main study began early in 1987 and will run over a nine year period. The mouse strains being used are B6DZF₁, DBA/2NNia, B6C3F₁, and C57BL/6, while rat strains are the Brown Norway, BNx F344F₁, and Fischer 344. Diets for mice are the NIH-31 and Emory Morris - 911, and for rats the NIH-31 and Masoro.

Dr. Allaben then described his own three part study on caloric restriction as a modulator of chemical carcinogenicity, effects on hepatic metabolism, DNA modification, and tumor incidences. The first part is concerned with the effects of caloric restriction on hepatic DNA modification by aflatoxin-B1. The second part will be to examine modulation of carcinogen metabolism by caloric restriction in control and aflatoxin-treated animals. Various xenobiotic metabolizing enzymes will be measured using specific substrates; both Phase 1 (cytochrome P-450 isozymes) and Phase 2 (conjugating enzymes) will be examined. The third part will be evaluating the modulation of neoplastic endpoints by caloric restriction using a liver tumor endpoint. Since the large study will use 40% caloric restriction, this degree of restriction is being used as well as groups with 30% restriction. The 40% restricted diet was considered to be nutritionally adequate. Dr. Allaben noted that tissues not used in their experiments would be available for use by other investigators at the Center. From the NIA part of the studies, animals will be available to investigators in the field of ageing research.

IX. Review of Chemicals Nominated for NTP Studies: There were 10 chemical nominations considered by the Board. All had been reviewed previously by the NTP Chemical Evaluation Committee (CEC). Dr. Gallo chaired the review. Dr. Dorothy Canter, NIEHS, Dr. William Allaben, NCTR, and Dr. Janet Haartz, NIOSH, CEC members, and Dr. Victor Fung, NIEHS, NTP Chemical Selection Coordinator, served as resource persons. Each Board member had been asked to serve as principal reviewer for one or two chemicals and following their presentation and discussion of each chemical, a motion was made and voted on by the members. During the review of one of the chemicals, ozone, presentations were made to the Board by Dr. Kathleen Nauss, Health Effects Institute, concerning the rationale for recommending NTP studies, and by Dr. Gary Boorman, NIEHS, pointing out the lack of adequate long-term animal studies on ozone.

The Board's recommendations including priority for study, and additional remarks and/or caveats for the 10 chemicals reviewed are summarized in Attachment 5.

NIEHS, P.O. Box 12233, Research Triangle Park, NC. 27709.

Date of meeting: January 25-26, 1988.

Place of meeting: Building 101 Conference Room, South Campus, NIEHS, Research Triangle Park, N.C.

Open: January 25, 9 a.m. to 12 noon.

Agenda: Discussion of the NIEHS budget, program policies and issues, recent legislation, and other items of interest.

Closed: January 25, 1 p.m. to recess; January 26, 9 a.m. to adjournment.

Closure reason: To review, discuss and evaluate individual grant applications.

(Catalog of Federal Domestic Assistance Program, Nos 13.112, Characterization of Environmental Health Hazards; 13.113, Biological Response to Environmental Health Hazards; 13.114, Applied Toxicological Research and Testing; 13.115, Biometry and Risk Estimation; 13.894, Resource and Manpower Development, National Institutes of Health)

Dated: November 6, 1987.

Betty J. Beveridge,

Committee Management Officer, NIH.

[FR Doc. 87-27089 Filed 11-24-87; 8:45am]

BILLING CODE 4140-01-M

Public Health Service

Drug Testing Provisions; Delegation of Authority

Notice is hereby given that in furtherance of the delegation by the Secretary of Health and Human Services on November 5, 1987, to the Assistant Secretary for Health, the Assistant Secretary for Health has delegated to the Administrator, Alcohol, Drug Abuse, and Mental Health Administration all the authorities delegated to the Assistant Secretary for Health under sections 503(a)(1)(A) and (B) and 503(c)(1) and (2) of the Drug Testing Provisions of the Supplemental Appropriations Act of 1987, Pub. L. 100-71, (5 U.S.C. 7301 Note), as amended hereafter. This delegation requires that in certifying agency drug testing plans the concurrence of an internal departmental Advisory Board must be obtained. It excludes the authority to promulgate regulations, submit reports to the Congress, and withhold certification of agency drug testing plans. Authority to redelegate is included other than the authority to certify agency drug testing plans.

The delegation to the Administrator, Alcohol, Drug Abuse, and Mental Health Administration became effective on November 18, 1987.

Dated: November 18, 1987.

Robert E. Windom,

Assistant Secretary for Health.

[FR Doc. 87-27149 Filed 11-24-87; 8:45 am]

BILLING CODE 4160-20-M

National Toxicology Program, Board of Scientific Counselors; Meeting

Pursuant to Public Law 92-463, notice is hereby given of a meeting of the National Toxicology Program (NTP) Board of Scientific Counselors, U.S. Public Health Service, in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina on December 14 and 15, 1987.

The meeting will be open to the public from 9:00 a.m. until adjournment on December 14. The preliminary agenda with approximate times are as follows:

9:00 a.m.-11:45 a.m.—Review of the Immunotoxicology Program, Division of Toxicology Research and Testing, NIEHS

12:45 p.m.-5:00 p.m.—Review of the Chemical Disposition Program, Division of Toxicology Research and Testing, NIEHS

The meeting on December 15 will be open to the public from 8:30 a.m. to 2:00 p.m. The preliminary agenda with approximate times are as follows:

8:30 a.m.-9:00 a.m.—Report of the Director, NTP

9:00 a.m.-10:00 a.m.—Patterns of Growth, Survival, and Tumor Trends in Rats and Mice from 1971-1983

10:15 a.m.-11:00 a.m.—NIEHS Dietary Restriction Studies in Rodents

11:00 a.m.-11:30 a.m.—NCTR Caloric Restriction Studies in Rodents

12:15 p.m.-2:00 p.m.—Review of Chemicals Nominated for NTP studies.

Ten chemicals will be reviewed. Six of the chemicals were evaluated by the NTP Chemical Evaluation Committee (CEC) on July 29, 1987, and are (with CAS Nos. in parentheses): (1) 1,4-Butanediol (110-63-4); (2) Carbon Disulfide (75-15-0); (3) Diethylene Glycol (111-46-6); (4) Dipropylene Glycol (25265-71-8); (5) Methylene Diphenyl Diisocyanate; and (6) Oxymetholone. Four of the chemicals were evaluated by the CEC on September 29, 1987, and are: (1) Heptachlor (76-44-8); (2) Heptachlor Epoxide (10214-57-3); (3) Ozone (10028-15-6); and (4) Primaclone (125-33-7).

In accordance with the provisions set forth in section 552b(c)(6) Title 5 U.S. Code and section 10(d) of Public Law 92-463, the meeting will be closed to the public on December 14 from 8:15 a.m. to 9:00 a.m. and on December 15 from 2:00

p.m. to 3:30 p.m. for further evaluation of research activities in the Immunotoxicology and Chemical Disposition Programs of the Systemic Toxicology Branch, including the consideration of personnel qualifications and performance, the competence of individual investigators, and similar items, the disclosure of which would constitute a clearly unwarranted invasion of personal privacy.

The Executive Secretary, Dr. Larry G. Hart, National Toxicology Program, P.O. Box 12233, Research Triangle Park, North Carolina 27709, telephone (919) 541-3971; FTS 629-3971, will have available a roster of Board members and expert consultants and other program information prior to the meeting, and summary minutes subsequent to the meeting.

Dated: November 2, 1987.

David P. Rail,

Director, National Toxicology Program.

[FR Doc. 87-27090 Filed 11-24-87; 8:45 am]

BILLING CODE 4140-01-M

Social Security Administration

Redelegations of Authority Under Equal Access to Justice Act

Under the Equal Access to Justice Act (EAJA), 5 United States Code 504, as reenacted and amended by Public Law 99-80 on August 5, 1985, and implementing regulations of the Department of Health and Human Services (the Department), published at 45 Code of Federal Regulations (CFR) Part 13, eligible individuals may be awarded attorney fees and other expenses when they prevail over the Department in administrative proceedings. These proceedings, which are called adversary adjudications, may result in reimbursement to involved individuals if they prevail in the proceedings and the Department's position in the proceedings was not substantially justified. A listing of Department proceedings covered by the EAJA appears at Appendix A of the implementing regulations.

When the EAJA was enacted in 1981, no Social Security Administration (SSA) proceedings were considered to be adversary adjudications. Congressional committee reports on the EAJA show that SSA's administrative process was exempted from provisions of the EAJA. Accordingly, SSA's proceedings were not included in Appendix A of the Department's EAJA regulations.

In 1982, SSA began the SSA Representative Project (SSARP) in five

AGENDA

BOARD OF SCIENTIFIC COUNSELORS
NATIONAL TOXICOLOGY PROGRAM

December 14 and 15, 1987

CONFERENCE CENTER, BUILDING 101, SOUTH CAMPUS

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES (NIEHS)

RESEARCH TRIANGLE PARK, NORTH CAROLINA

Monday, December 14, 1987CLOSED MEETING

8:15 a.m. - 9:00 a.m.	Evaluation of Personnel in the Immunotoxicology and Chemical Disposition Programs, NIEHS Systemic Toxicology Branch	Board and Consultants
-----------------------	---	-----------------------

OPEN MEETING

Review of Immunotoxicology Program, Systemic Toxicology Branch, Division of Toxicology Research And Testing, NIEHS*

9:00 a.m. - 9:15 a.m.	Branch Overview	Dr. B. Schwetz
9:15 a.m. - 10:35 a.m.	Background History and Program Overview	Dr. M. Luster
	Contracts Review	Dr. M. Luster
	Studies of B Lymphocyte Maturation Using Chemical Probes	Dr. J. Blank
	Examination of PMN Function in Immunotoxicology	Dr. M. Ackermann
10:50 a.m. - 12:00 noon	Overview of Lung Immunotoxicology Program	Dr. G. Rosenthal
	In-House Research Efforts and Future Directions	Dr. M. Luster
	Discussion	

Review of Chemical Disposition Program, Systemic Toxicology
Branch, Division of Toxicology Research and Testing, NIEHS

12:45 p.m. - 3:00 p.m.	Introduction	Dr. H. Matthews
	Metabolism, Aging and Toxicity	Dr. L. Birnbaum
	Xenobiotic Metabolism	Dr. L. Burka
	Butoxyethanol Induced Hematotoxicity, and Mechanisms of Forestomach Toxicity	Dr. B. Ghanayem
3:00 p.m. - 3:45 p.m.	Viewing of Posters	
3:45 p.m. - 4:45 p.m.	Contract and Interagency Agreement Reviews	
	A. Chemical Disposition	Dr. H. Matthews
	B. Inhalation Studies	Dr. L. Birnbaum
	C. Human Metabolism Studies	Dr. L. Burka
	Conclusions and Future Plans	Dr. H. Matthews

* Posters of research projects in the Immunotoxicology and Chemical Disposition Programs will be available for public viewing in the Main Lobby, Building 101.

Tuesday, December 15, 1987

OPEN MEETING

8:30 a.m. - 9:00 a.m.	Report of the Director, NTP	Dr. D. Rall, NIEHS
9:00 a.m. - 9:30 a.m.	NIEHS/NTP Concept Review: Development of Mutagenesis Assays Using Transgenic Mice	Dr. J. Mason, NIEHS
9:30 a.m. - 10:30 a.m.	Patterns of Growth, Survival and Tumor Trends in Rats and Mice from 1971-1983	Dr. G. Rao, NIEHS
10:45 a.m. - 11:30 a.m.	NIEHS Dietary Restriction Studies in Rodents	Dr. K. Abdo, NIEHS
11:30 a.m. - 12:00 noon	NCTR Caloric Restriction Studies in Rodents	Dr. W. Allaben and Dr. A. Turturro, NCTR

CLOSED MEETING

12:15 p.m. - 2:00 p.m.	Evaluation of Programs and Personnel in the Immuno- toxicology and Chemical Disposition Programs, NIEHS Systemic Toxicology Branch	Board and Consultants
------------------------	--	-----------------------

OPEN MEETING

2:15 p.m. - 4:00 p.m.	Review of Chemicals Nominated for NTP Studies	Board Dr. D. Canter, NIEHS
-----------------------	--	-------------------------------

Adjourn

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS

December 14-15, 1987

Dr. Michael A. Gallo (3/89)
Assoc. Professor, Dir. of Toxicology
Dept. of Environmental and Comm. Medicine
UMDNJ - Robert Wood Johnson Med. School
675 Hoes Lane
Piscataway, New Jersey 08854-5635

Dr. Richard A. Griesemer (3/91)
Director, Biology Division
Oak Ridge National Laboratory
P. O. Box Y
Oak Ridge, Tennessee 37830

Dr. Robert A. Scala (3/90)
Senior Scientific Advisor
Research and Environmental
Health Division
Exxon Biomedical Sciences
Mettlers Road, CN 2350
East Millstone, New Jersey 08875-2350

Dr. John B. Little, M.D. (3/91)
Professor of Radiobiology
Department of Cancer Biology
Harvard School of Public Health
Boston, Massachusetts 02115

Dr. Frederica Perera (4/89)
Columbia University
School of Public Health
Division of Environmental Sciences
60 Haven Avenue, B-109
New York, New York 10032

Dr. Adrienne E. Rogers (3/90)
Professor, Department of Pathology
Boston University School of
Medicine
80 East Concord Street, L-804
Boston, Massachusetts 02118

Dr. Arthur C. Upton, M.D. (3/91)
Director, Institute of
Environmental Medicine
New York University Medical Center
550 First Avenue
New York, New York 10016

AD HOC REVIEWERS FOR NTP BOARD OF
SCIENTIFIC COUNSELORS REVIEW OF
SYSTEMIC TOXICOLOGY BRANCH PROGRAMS

December 14-15, 1987

Immunotoxicology Program

Dr. John B. Barnett
Department of Microbiology and Immunology
University of Arkansas Medical School
4301 West Markham Street
Little Rock, Arkansas 72205

Dr. Daniel Wierda
Department of Pharmacology and Toxicology
West Virginia University Medical Center
Morgantown, West Virginia 26506

Chemical Disposition Program

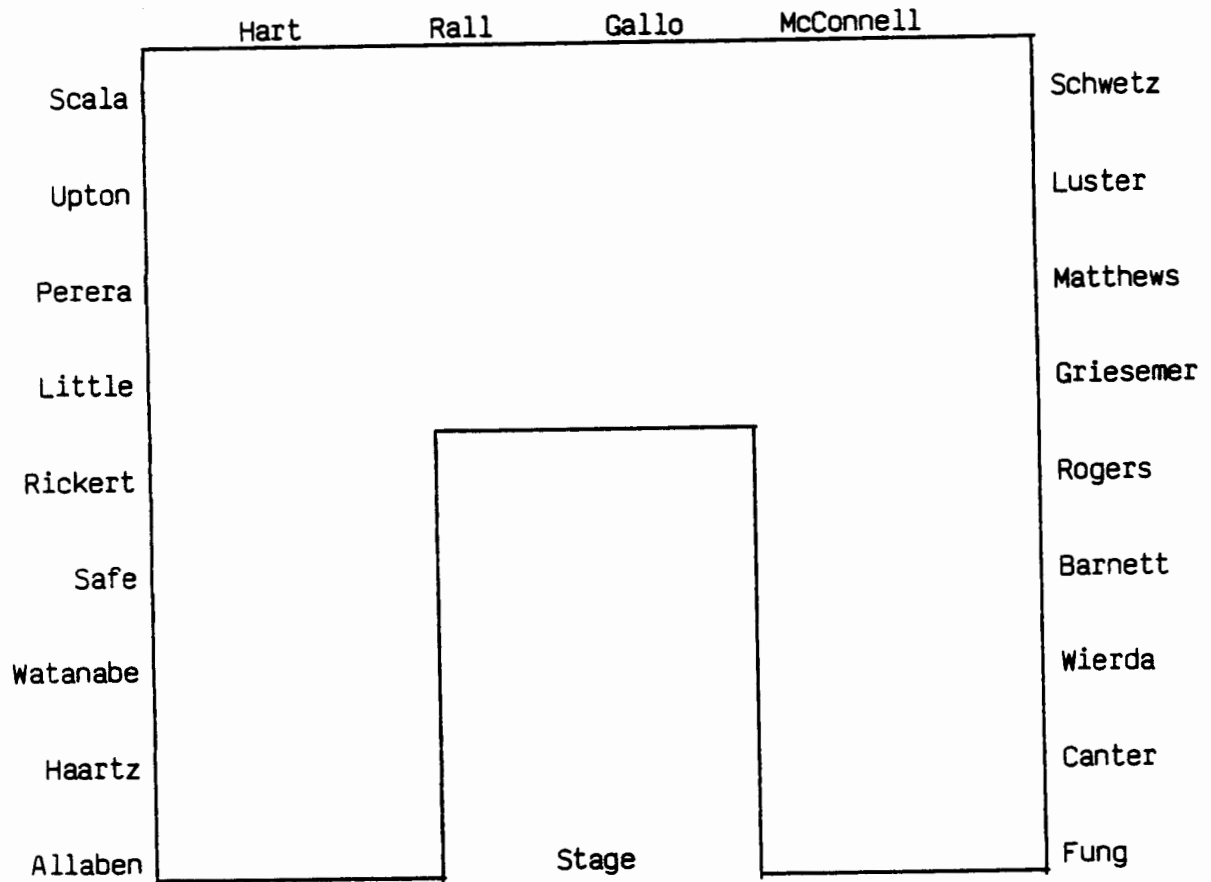
Dr. Douglas Rickert
Chemical Industry Institute of Toxicology
Post Office Box 12137
Research Triangle Park, North Carolina 27709

Dr. Stephen Safe
College of Veterinary Medicine
Texas A & M University
College Station, Texas 77843

Dr. Philip Watanabe
Toxicology Department
Dow Chemical Company
1803 Building
Midland, Michigan 48640

NTP BOARD OF SCIENTIFIC COUNSELORS MEETING

Conference Center, Building 101
National Institute of Environmental Health Sciences
Research Triangle Park, North Carolina
December 14-15, 1987



December 14, 1987
Dr. James M. Mason and
Dr. Lawrence R. Boone
Cellular and Genetic
Toxicology Branch

National Toxicology Program
Concept Review

TITLE: Development of Mutagenesis Assays Using Transgenic Mice.

PERIOD AND TYPE OF AWARD: 5 Years - Research and Development Contract,
Two or more awards are anticipated.

FUNDING MECHANISM: Competitive Award

OBJECTIVE: To develop one or more in vivo mutagenesis assay systems to detect and quantitate gene mutations at a precisely defined target sequence in somatic, and possibly germ, cells of mice exposed to a test chemical. Such assays will provide a means to detect analyze organ and tissue specific mutagenesis and study associations with chemical disposition, toxicity, and carcinogenicity. This will provide an opportunity to investigate the relationship between a chemical's mutagenic activity in vivo and its carcinogenicity. Ultimately, such systems may provide an in vivo short term assay with predictive value for carcinogenicity. Such assays may also be amenable to use in measuring in vivo germ cell mutagenesis.

BACKGROUND: For several years, in vitro mutagenesis assays have been used in an effort to identify potential carcinogens. The use of these tests is predicated on the somatic mutation theory of carcinogenesis and early observations of a high correlation coefficient between mutagenesis test results in vitro and carcinogenesis tests results in vivo. In more recent comparisons, however, the agreement between in vitro test results and carcinogenesis was no better than 60% for four different in vitro assays (Tennant et al. 1987). This raises a number of questions about the extent to which one can expect in vitro assays to predict the outcome of a process as complex as carcinogenesis. The ability to analyze gene mutation in the whole animal, in the tissues and organs at risk for developing chemically induced neoplasms, would greatly increase our understanding of the relevance of the mutagenicity of individual chemicals to the mechanisms of carcinogenesis. For example, carcinogens have been identified that are not mutagenic in vitro, but apparently induce novel H-ras mutations at the site of tumorigenesis (Reynolds et al. 1987).

In recent years convenient mutagenesis assay systems have been developed in which precisely defined target genes are carried on a shuttle vector that can be mutated in cultured mammalian cells and transferred to bacterial cells for scoring and detailed analysis, including DNA sequencing. Often, a bacterial gene such as lacI (DuBridge et al. 1987) or supF (Seidman et al. 1985) are used as forward mutation targets. The prospect of extending this to a whole animal somatic cell mutagenesis assay stems from important recent advances in molecular biology, including the development of transgenic mice. In this technique recombinant DNA-derived genes are introduced into the mouse germ

line where they are inherited in Mendelian fashion (Brinster et al. 1981, Costantini and Lacy 1981, Gordon et al. 1980, Wagner et al. 1981). The transcriptional activity of the transgene is determined in part by the choice of promoter/enhancer sequences included in the construct and in part by the genomic milieu determined by the site of integration, which is random. Transgenic mice have been used successfully in experiments to study control of gene expression (Westphal et al. 1985), gene therapy experiments (Readhead et al. 1987), and as a means to study sub-threshold neoplastic states induced by certain activated oncogenes (Stewart et al. 1984). This technique allows one to engineer genetically a mutation target to suit a specific need and have that target available in every cell of the test animal. Having the transgene integrated into the host chromosome in an authentic chromatin structure is much more appropriate as a mutation target than the episomal shuttle vector of many current in vitro mammalian cell mutagenesis systems.

GOALS: There are several programmatic goals that can be met through the use of an in vivo mutagenesis assay system. While each of these may have its own set of requirements, several of the goals may be addressable using the same assay system. The goals of the project and the general form of the respective assay systems are outlined below.

(1) Rapid screen for chemical mutagens: Such an assay may involve a forward mutation assay in order to identify a broad range of mutational events. A selectable marker could be used as a target to increase the efficiency of recovery or a histochemical, immunohistochemical or morphological assay might be scored in intact tissues.

(2) Detailed molecular analysis of induced mutations: The target should be amenable to convenient scoring in a bacterial host and should be relatively small to make detailed analysis, including sequencing, easy. Target gene and analysis strategies similar to the shuttle vector systems are likely to be appropriate.

(3) Germ cell mutation assay: Somatic cell mutagenesis assays may be altered slightly to target germinal tissue with a specific promoter sequence or by separating germ cells from the somatic cells in the gonads after chemical treatment.

(4) Assay for mutations induced in specific tissues: To derive maximum information on the relation between tissue specific mutagenesis and carcinogenesis, especially in regard to the Branch's goal of predicting potential tumorigenicity, the B6C3F1 mouse should be used. Constructs carrying oncogenes may be useful for detecting and characterizing activation of oncogenes by mutagenic or nonmutagenic carcinogens. Because of technical difficulties in constructing transgenic mice, it may be advisable to develop appropriate assays in other strains of mice before incorporating them into the B6C3F1 hybrid.

With these goals in mind, we wish to solicit proposals to construct suitable recombinant DNA clones containing mutation targets, develop and characterize transgenic strains carrying the mutation target and perform a limited analysis with known mutagens and carcinogens to evaluate the utility of the system.

REFERENCES:

- Brinster, R.L., H.Y. Khan, M.E. Trumbauer, A.W. Seneear R. Warren and R.D. Palmiter, 1981, Somatic expression of herpes thymidine kinase in mice following injection of a fusion gene into eggs. *Cell* 27: 223-231.
- Costantini, F. and E. Lacy, 1981, Introduction of a rabbit B-globin gene into the mouse germline. *Nature* 294: 92-94.
- DuBridge, R. B., P. Tang, H. C. Hsia, P.-M. Leong, J. H. Miller, and M. P. Calos, 1987, Analysis of mutation in human cells by using an Epstein-Barr virus shuttle system. *Mol. Cell. Biol.* 7: 379-387.
- Gordon, A.W., G.A. Scangos, D.J. Plotkin, J.A. Barbosa and F.H. Ruddle, 1980, Genetic transformation of mouse embryos by microinjection of purified DNA. *Proc. Natl. Acad. Sci. USA* 77: 7380-7384.
- Readhead, C., B. Popko, N. Takahashi, H. D. Shine, R. A. Saavedra, R. L. Sidman and L. Hood, 1987, Expression of a myelin basic protein gene in transgenic shiverer mice: correction of the dysmyelinating phenotype. *Cell* 48: 703-712.
- Reynolds, S. H., S. J. Stowers, R. M. Patterson, R. P. Maronpot, S. A. Aaronson, M.W. Anderson, 1987, Activated oncogenes in B6C3F1 mouse liver tumors: implications for risk assessment. *Science* 237: 1309-1316.
- Seidman, M. M., K. Dixon, A. Razzaque, R. J. Zagursky, and M. L. Berman, 1985, A shuttle vector plasmid for studying carcinogen-induced point mutations in mammalian cells. *Gene* 38: 233-237.
- Stewart, T. A., P. K. Pattengale, and P. Leder, 1984, Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* 38: 627-637.
- Tennant, R.W., B.H. Margolin, M.D. Shelby, E. Zeiger, J.K. Haseman, J. Spalding, W. Caspary, M.A. Resnick, S. Stasiewicz, B. Anderson, and R. Minor, 1987, Prediction of chemical carcinogenicity in rodents from in vitro genetic toxicology assays. *Science* 236: 933-941.
- Wagner, E.F., T.A. Stewart and B. Mintz, 1981, The human B-globin gene and a functional thymidine kinase gene in developing mice. *Proc. Natl. Acad. Sci. USA* 78: 5016-5020
- Westphal, H., P. A. Overbeek, J. S. Khillan, A. B. Chepelinsky, A. Schmidt, K. A. Mahon, K. E. Bernstein, J. Piatigorsky, and B. De Crombrughe, 1985, Promoter sequences of murine @A crystallin, murine @2(I) collagen or of avian sarcoma virus genes linked to the bacterial chloramphenicol acetyl transferase expression in transgenic mice. *CSHSQB* 50: 411-416.

GROWTH, BODY WEIGHT, SURVIVAL AND TUMOR TRENDS
IN F344 RATS AND B6C3F1 MICE FROM 1971 TO 1983

G.N. Rao, D.D. Crawford and J.K. Haseman

RATS

<u>YEAR</u>	<u>NUMBER OF STUDIES</u>	<u>LABORATORY</u>	<u>NUMBER OF STUDIES</u>
1971	3	BC	8
1972	22	DW	2
1973	42	FC	23
1974	10	GS	9
1975	4	HZ	6
1976	9	LB	32
1977	13	MA	27
1978	12	PA	1
1979	11	PR	8
1980	10	SO	25
1981	8	SR	3

YEAR	LABORATORY	NUMBER OF STUDIES	YEAR	LABORATORY	NUMBER OF STUDIES
1971	LB	2	1977	BC	3
	MA	1		FC	1
				MA	7
1972	LB	9		SO	2
	MA	6	1978	BC	2
	SO	7		GS	2
1973	DW	2		LB	1
	FC	17		MA	2
	LB	12		PA	1
	MA	4		SO	4
	SO	7	1979	FC	1
1974	FC	4		GS	5
	LB	4		MA	3
	MA	2		SO	2
1975	HZ	1	1980	GS	2
	SO	1		HZ	2
1976	BC	1		PR	5
	HZ	1		SO	1
	LB	4	1981	BC	2
	MA	2		PR	3
	SO	1		SR	3

MALE RATS

GROWTH CURVES

BODY WEIGHT

SURVIVAL

HEMATOPOIETIC SYSTEM TUMORS

ANTERIOR PITUITARY TUMORS

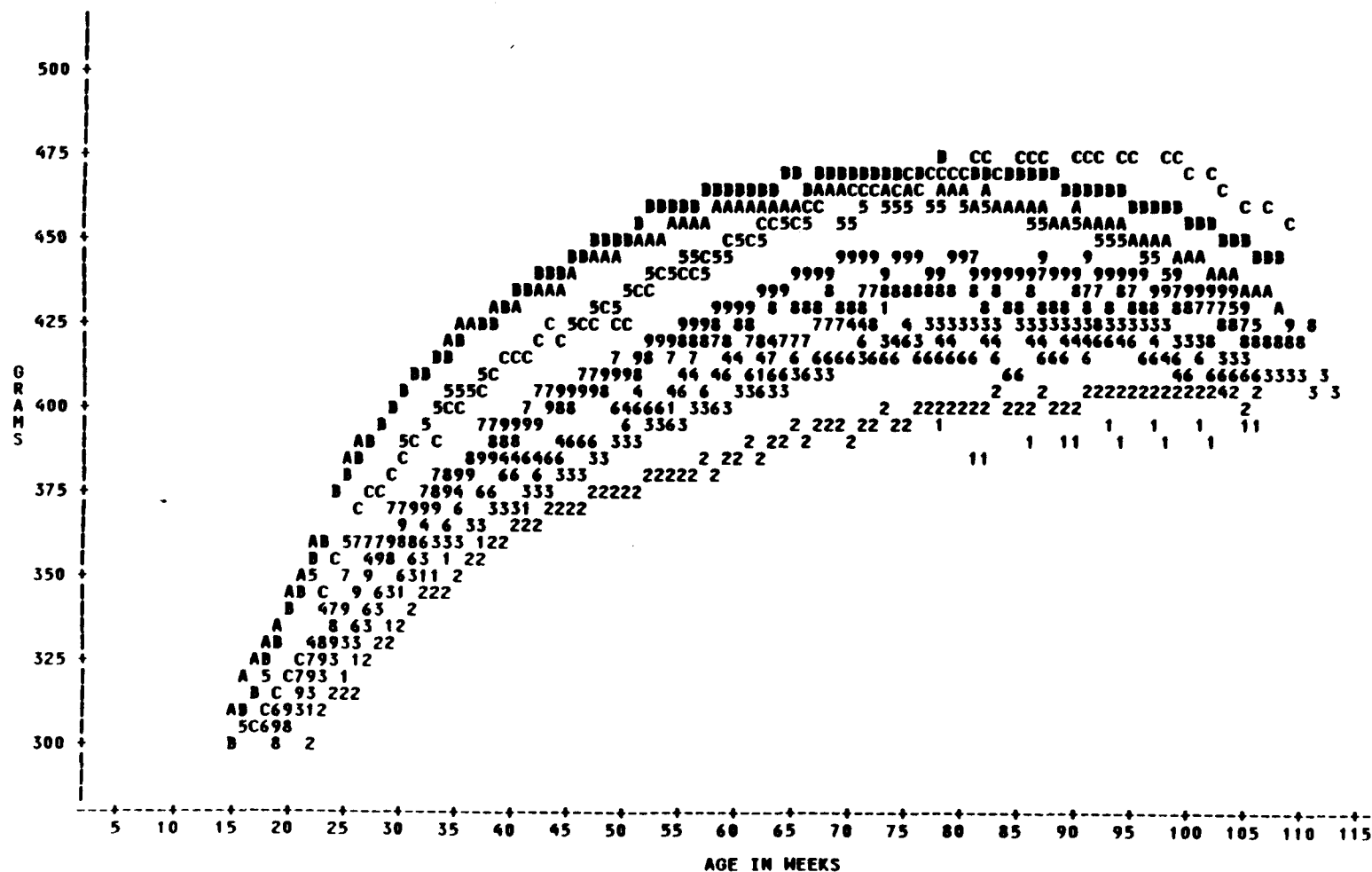
ADRENAL PHEOCHROMOCYTOMAS

THYROID C-CELL TUMORS

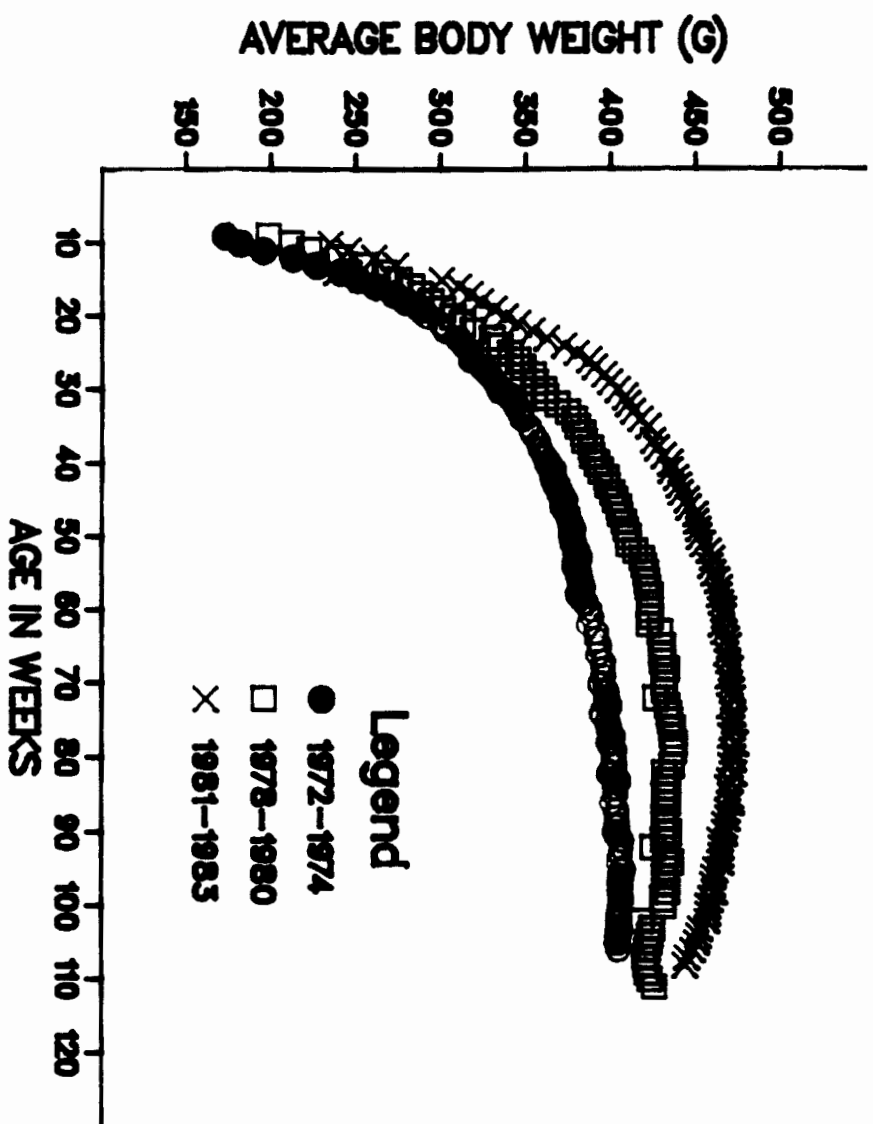
CARCINOGENESIS BIOASSAY DATA SYSTEM, YEARS OF BIRTH 1971-1982
 PLOT OF 9-WEEK MOVING AVERAGE WEIGHTS, YEARS OF BIRTH 1971-1982

SYMBOL CORRESPONDS TO YEAR AS FOLLOWS: 1971=1, 1972=2, ..., 1980=A, 1981=B, 1982=C

SPECIES=RAT STRAIN=F344 SEX=M

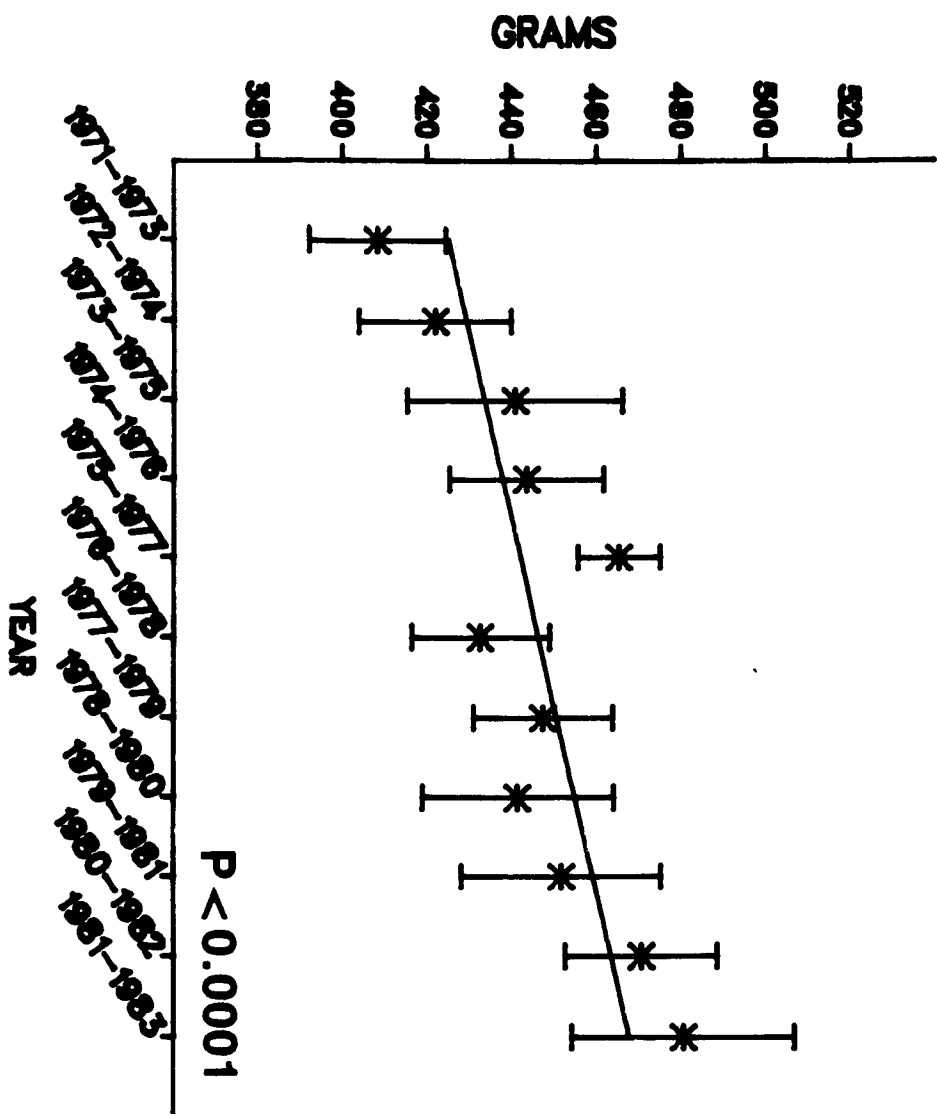


MALE RATS

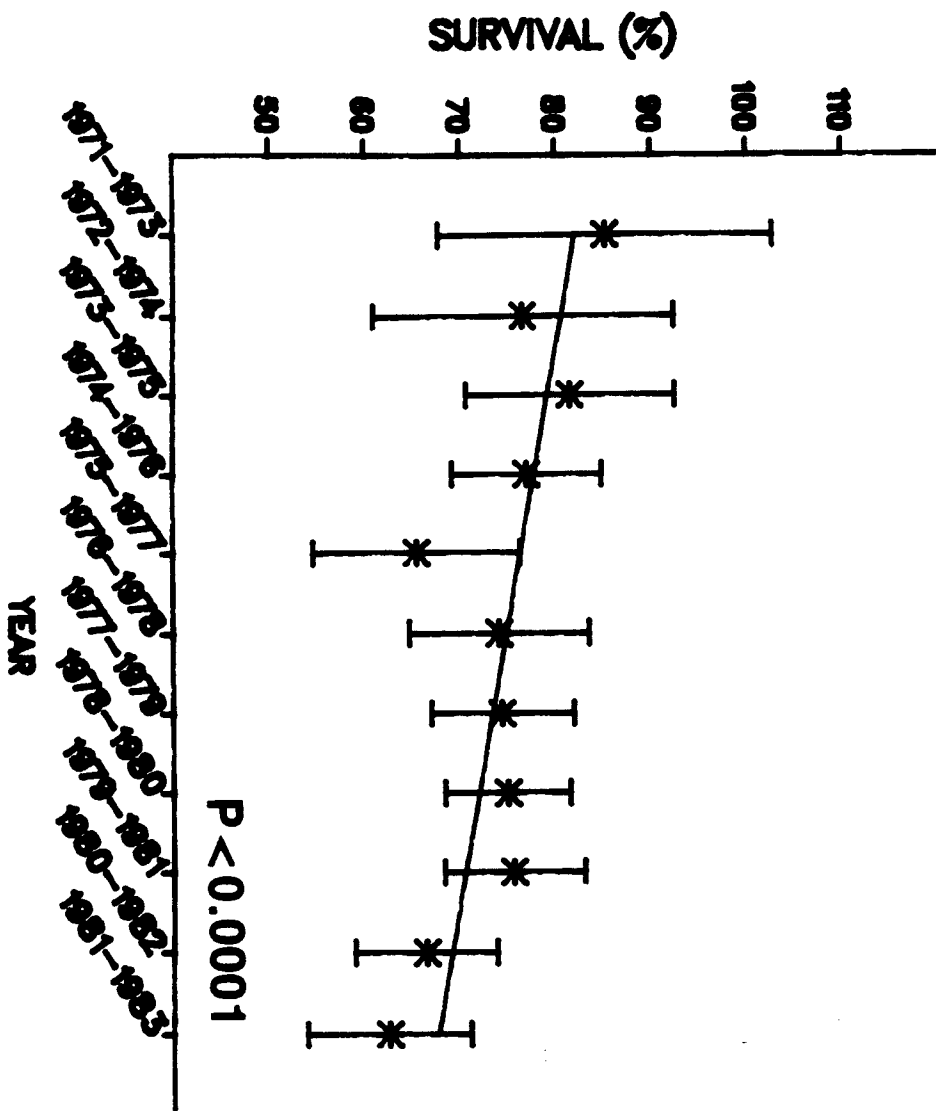


MALE RATS

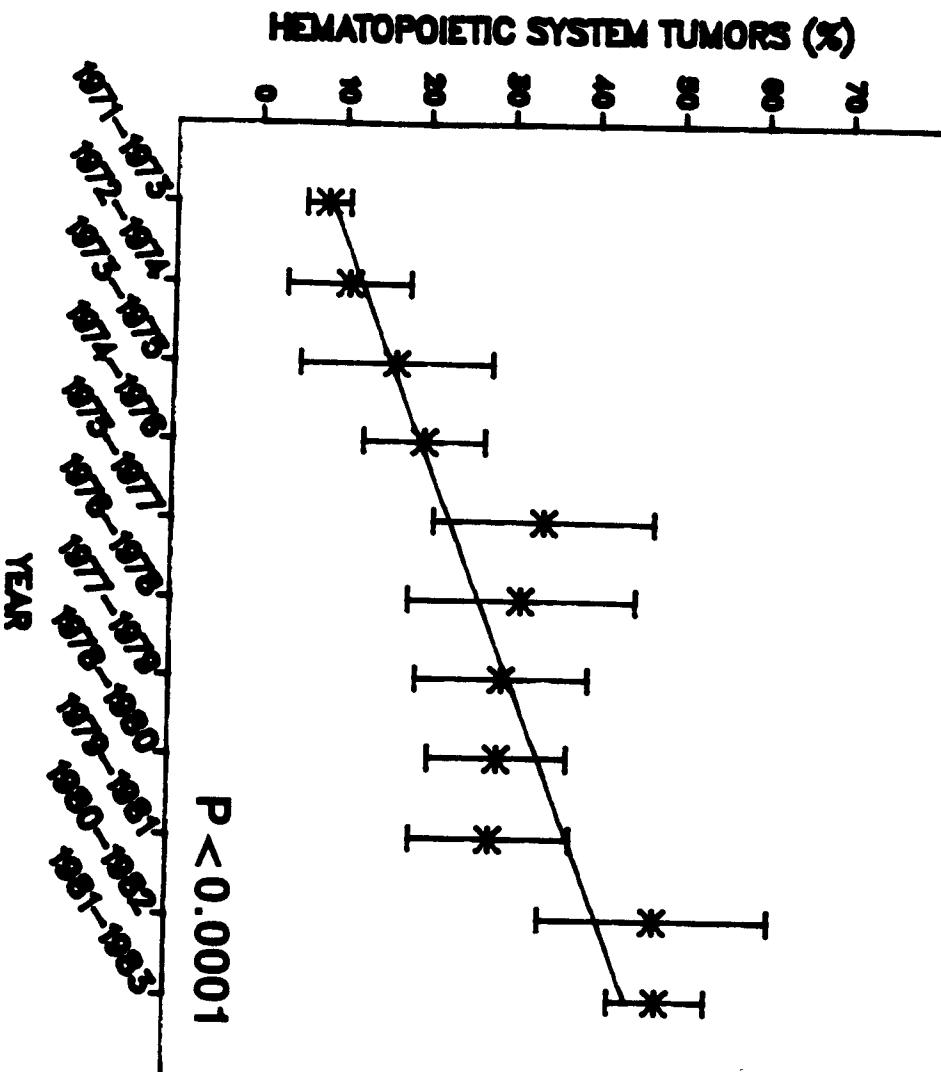
MAXIMUM MEAN BODY WEIGHT



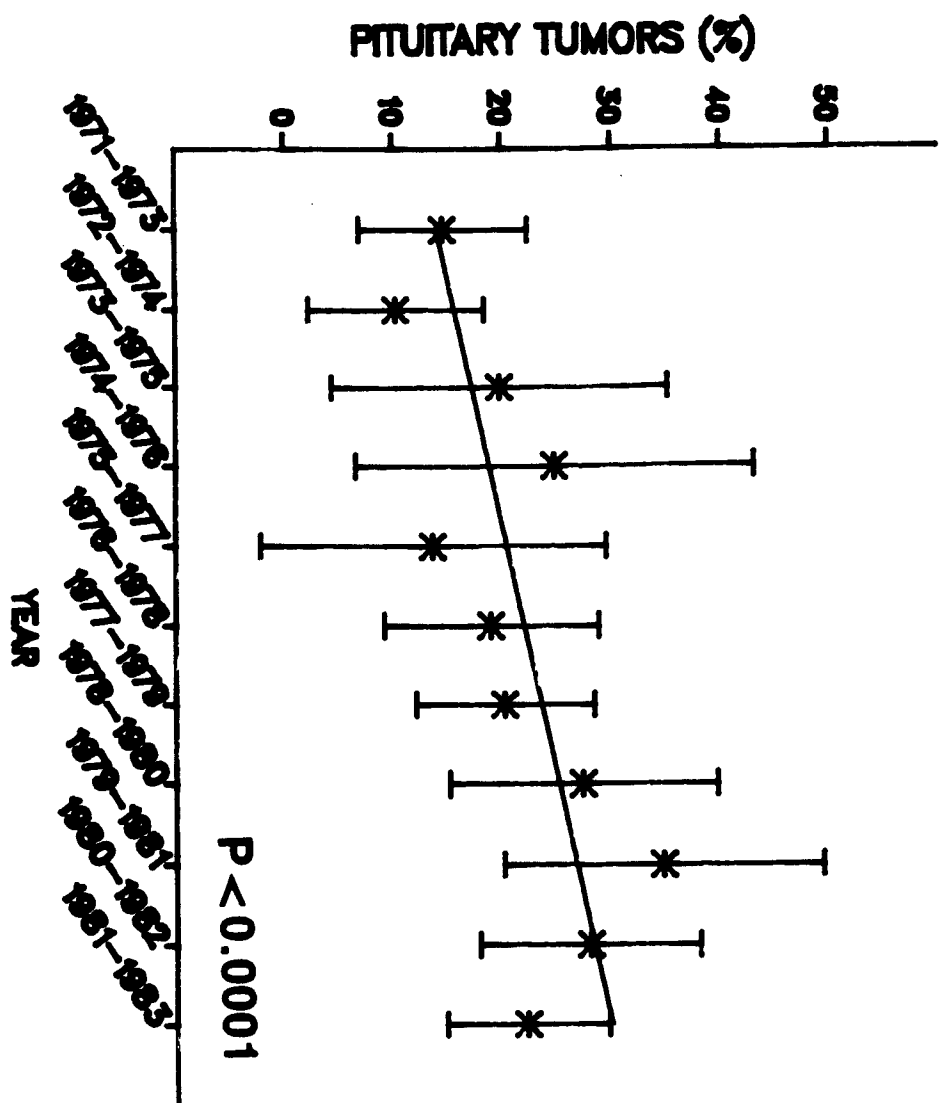
MALE RATS



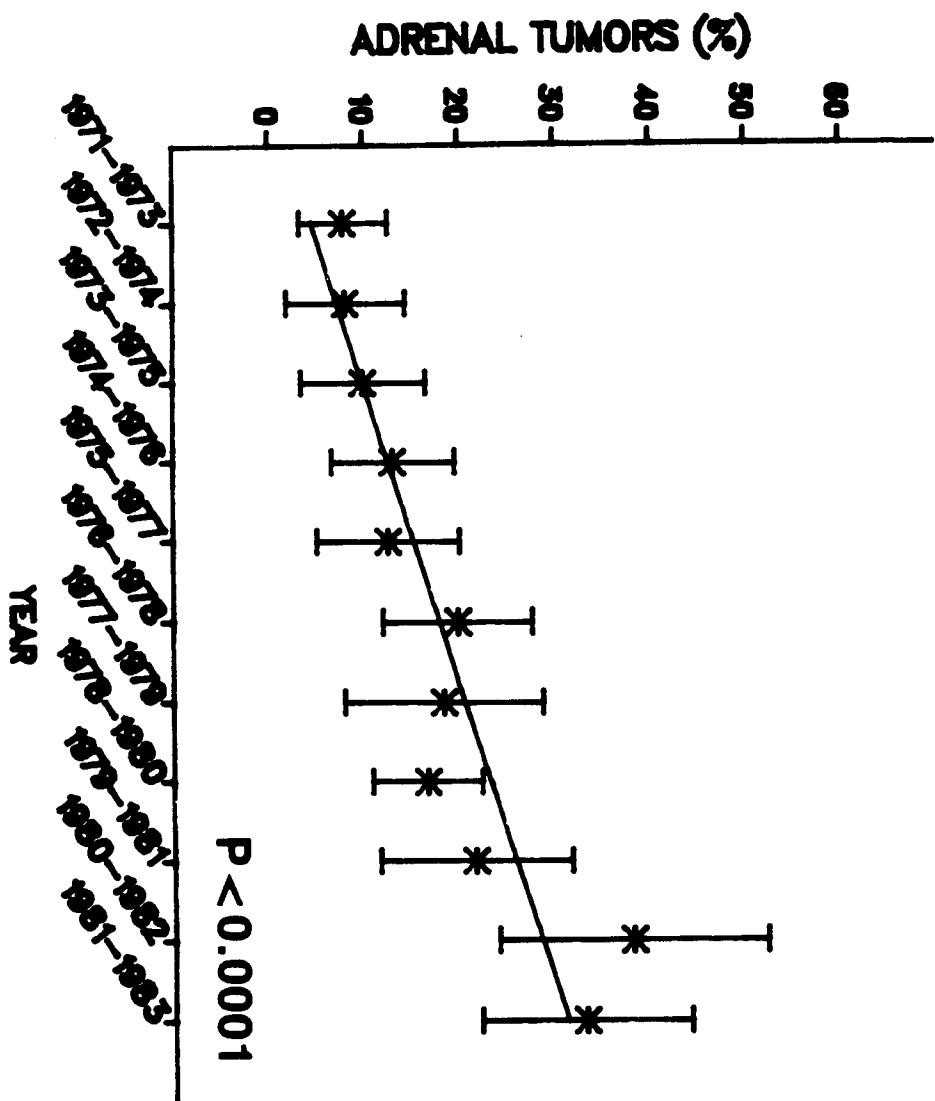
MALE RATS



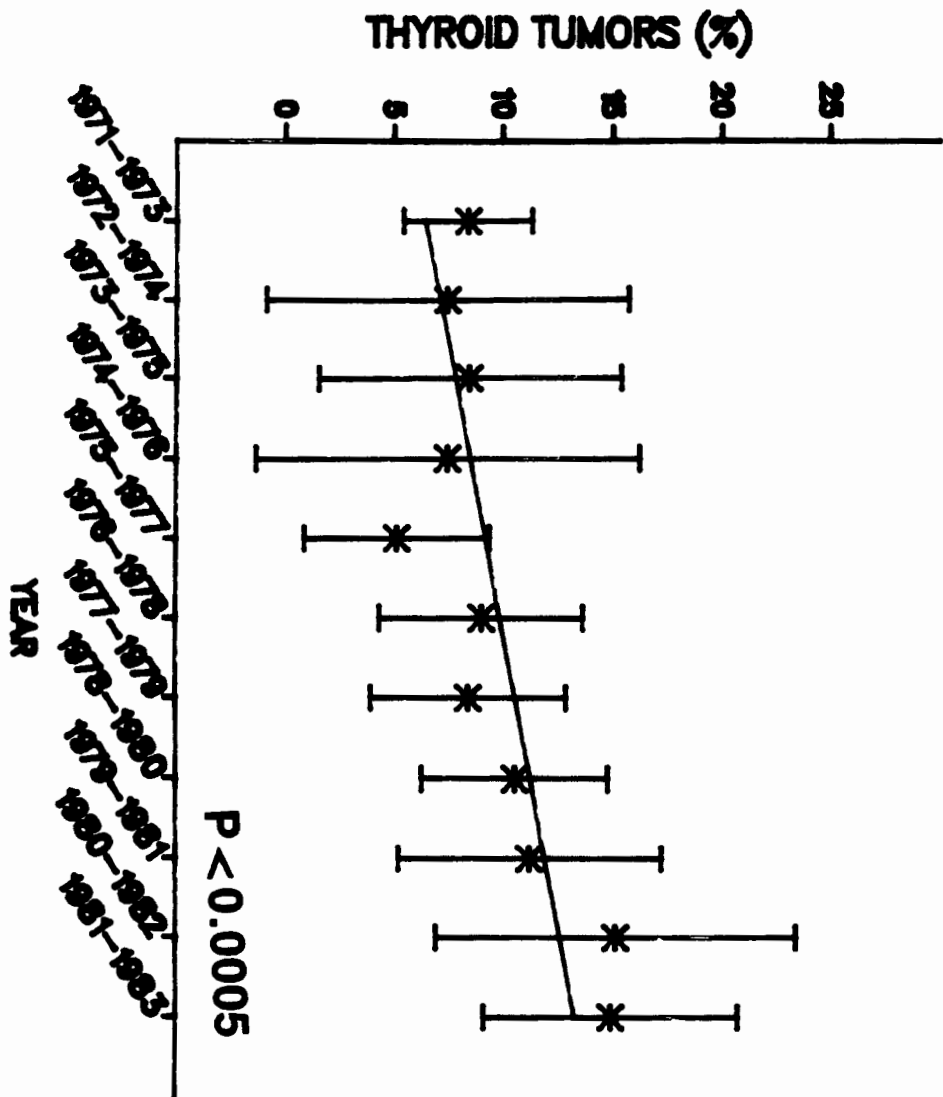
MALE RATS



MALE RATS



MALE RATS



FEMALE RATS

GROWTH CURVES

BODY WEIGHT

SURVIVAL

HEMATOPOIETIC SYSTEM TUMORS

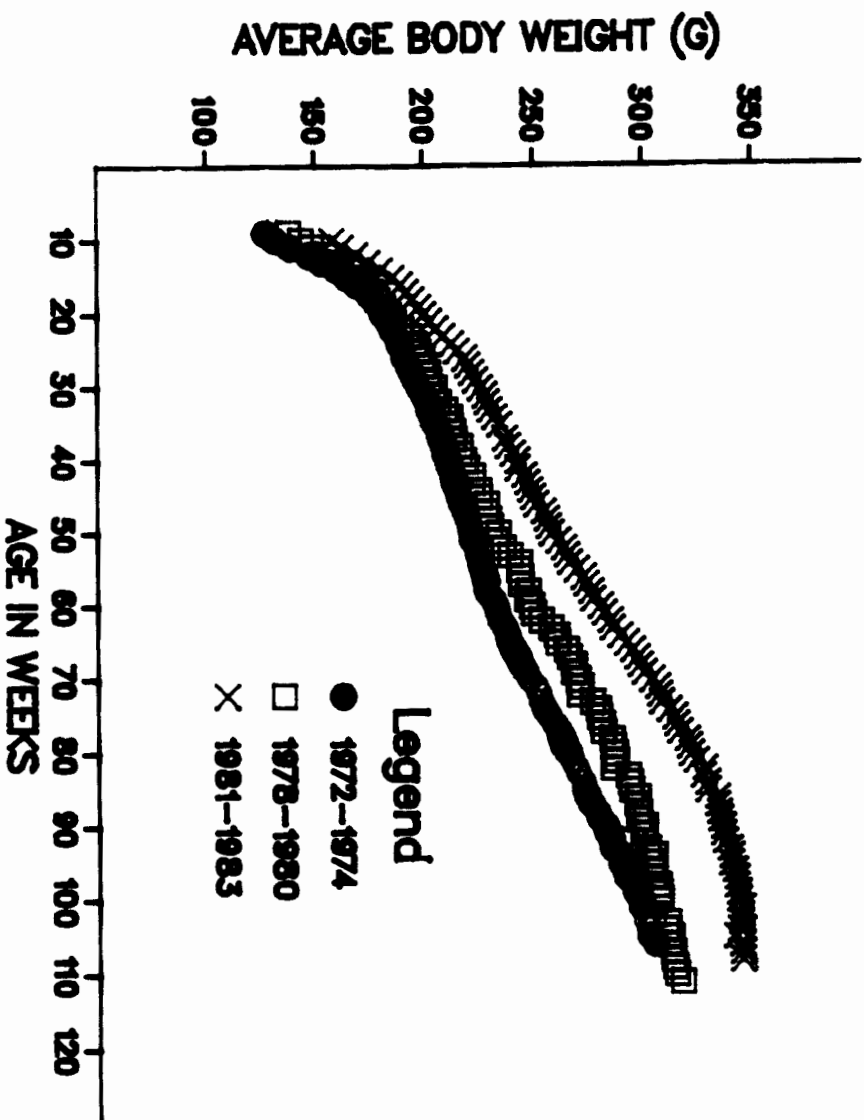
ANTERIOR PITUITARY TUMORS

THYROID C-CELL TUMORS

MAMMARY GLAND TUMORS

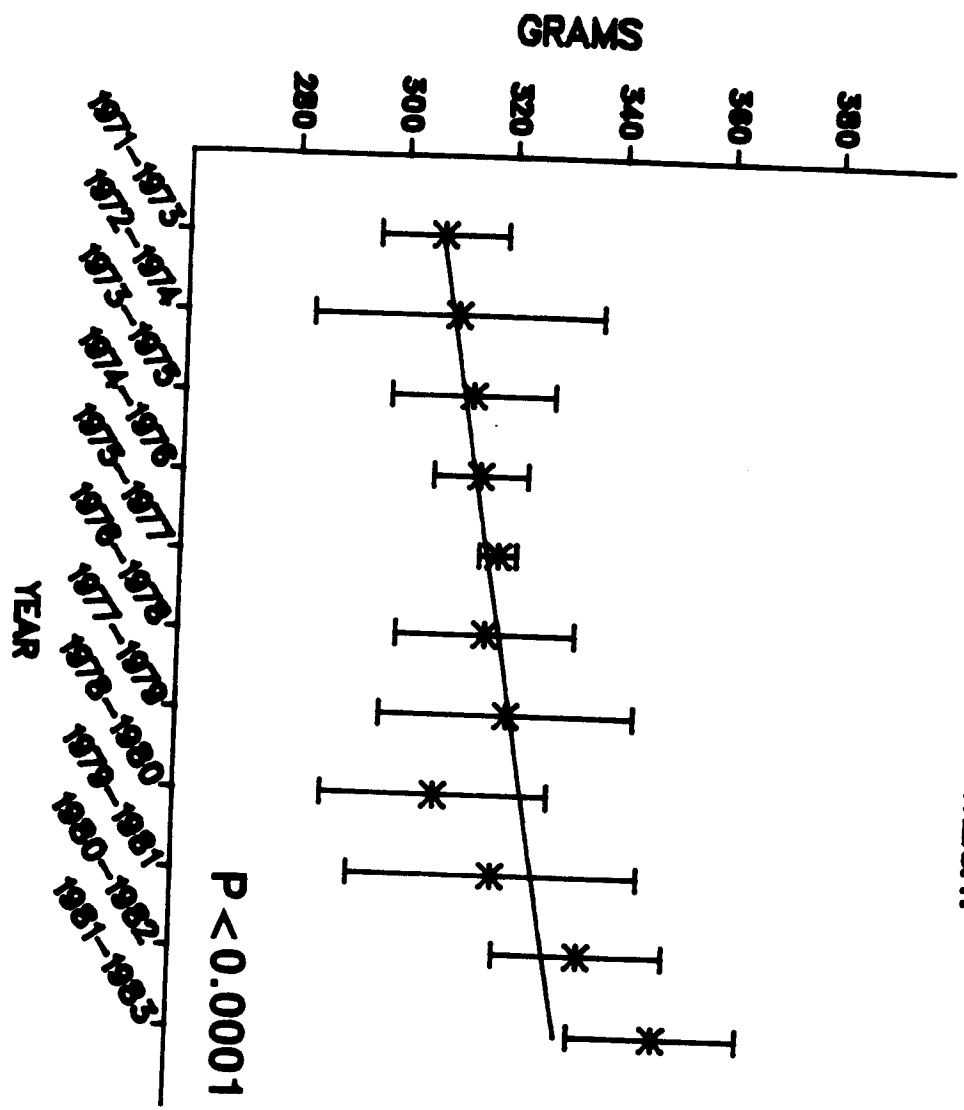
ENDOMETRIAL STROMAL POLYPS

FEMALE RATS

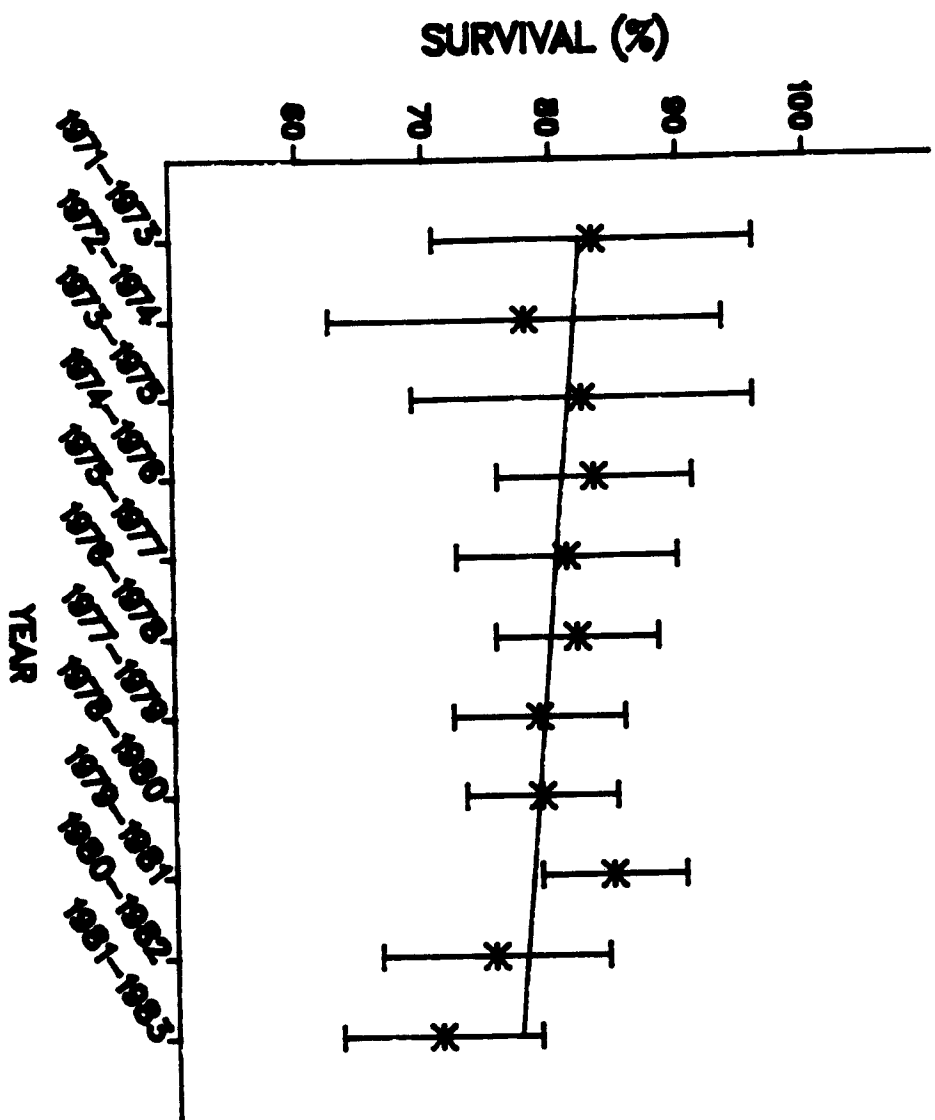


FEMALE RATS

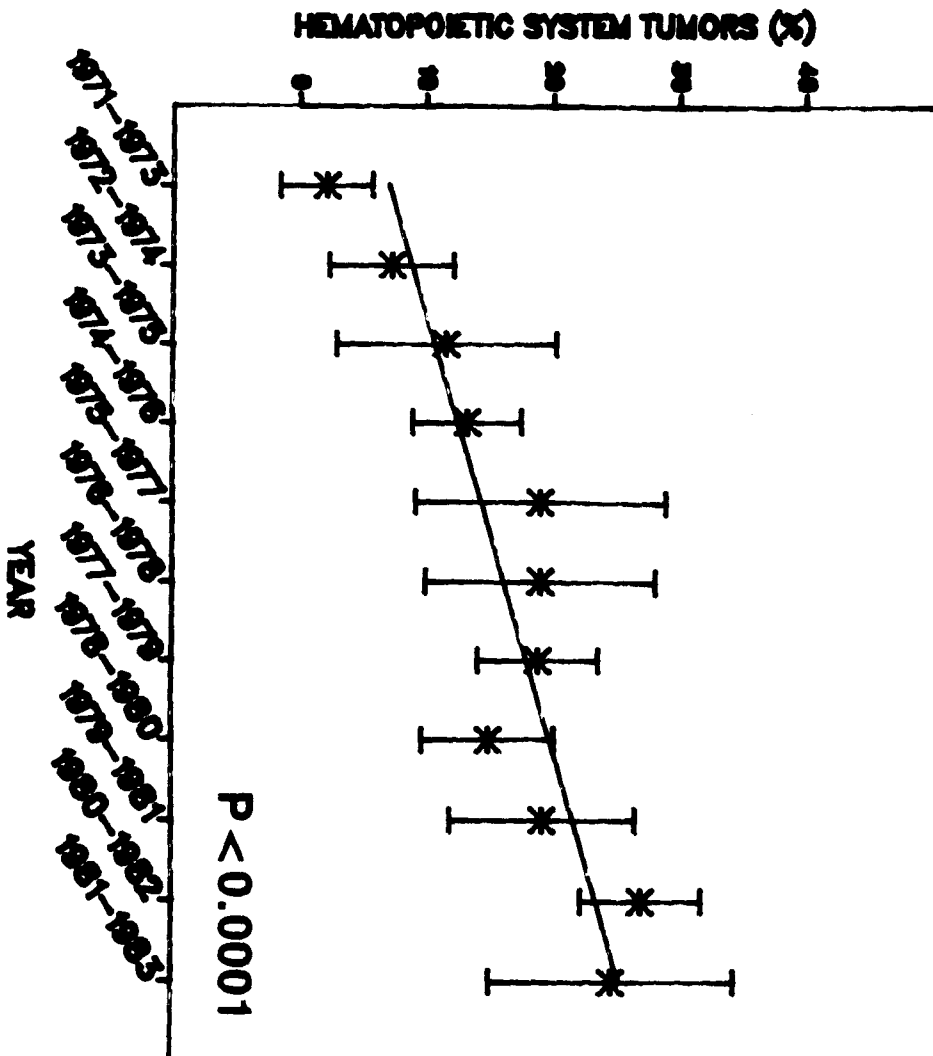
MAXIMUM MEAN BODY WEIGHT



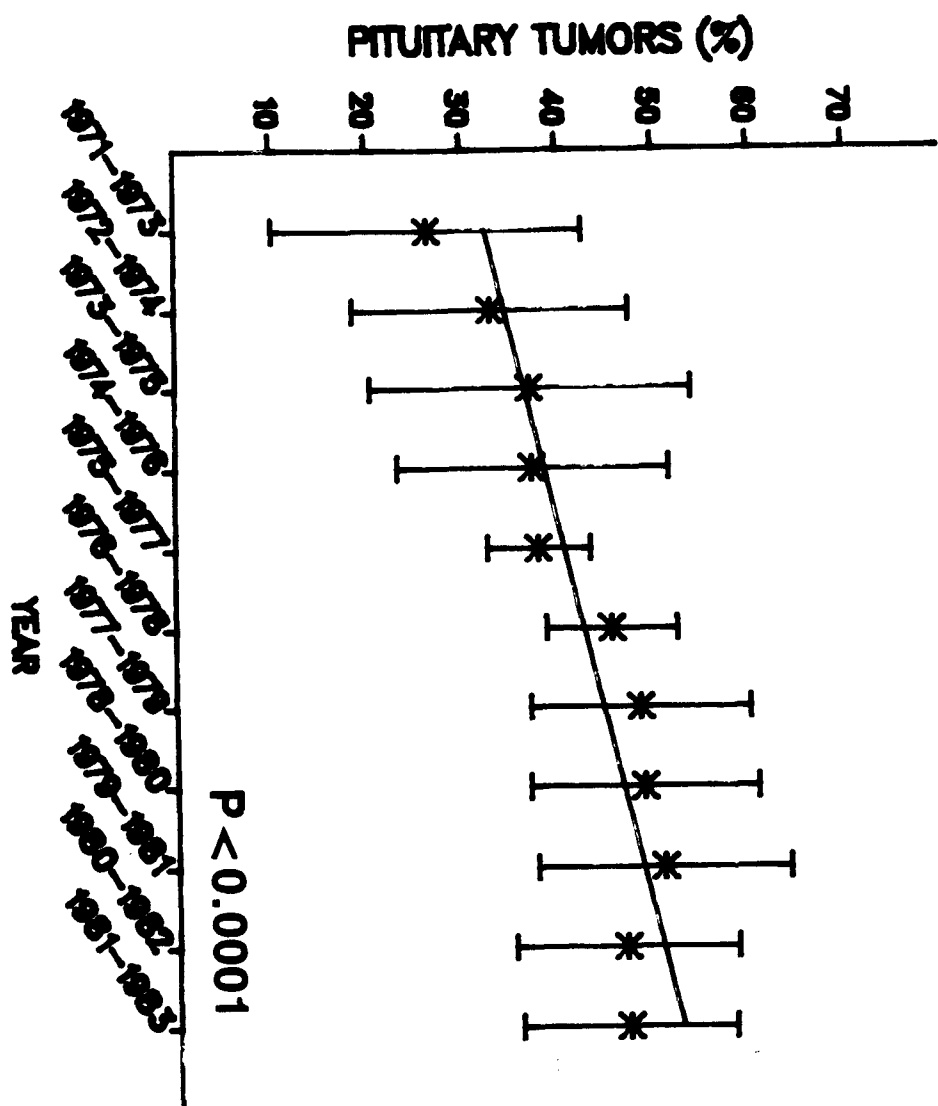
FEMALE RATS



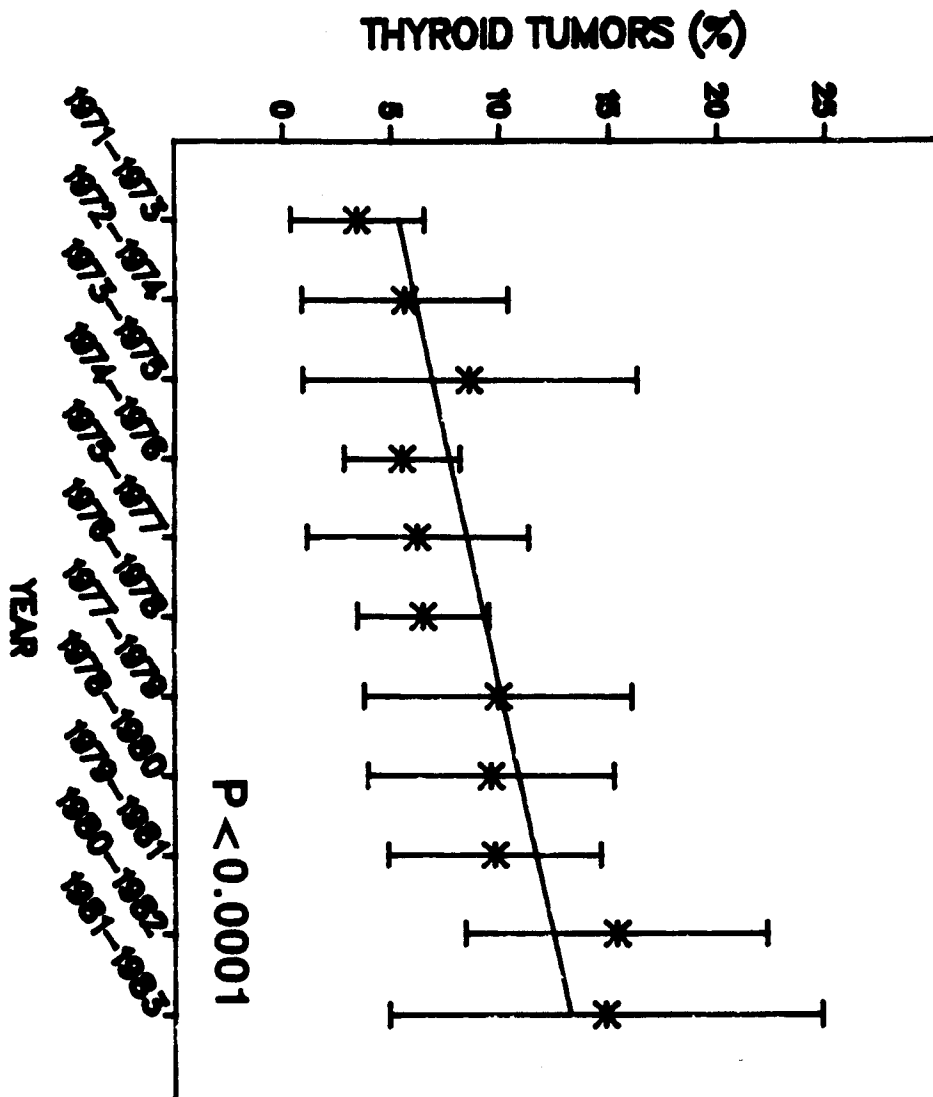
FEMALE RATS



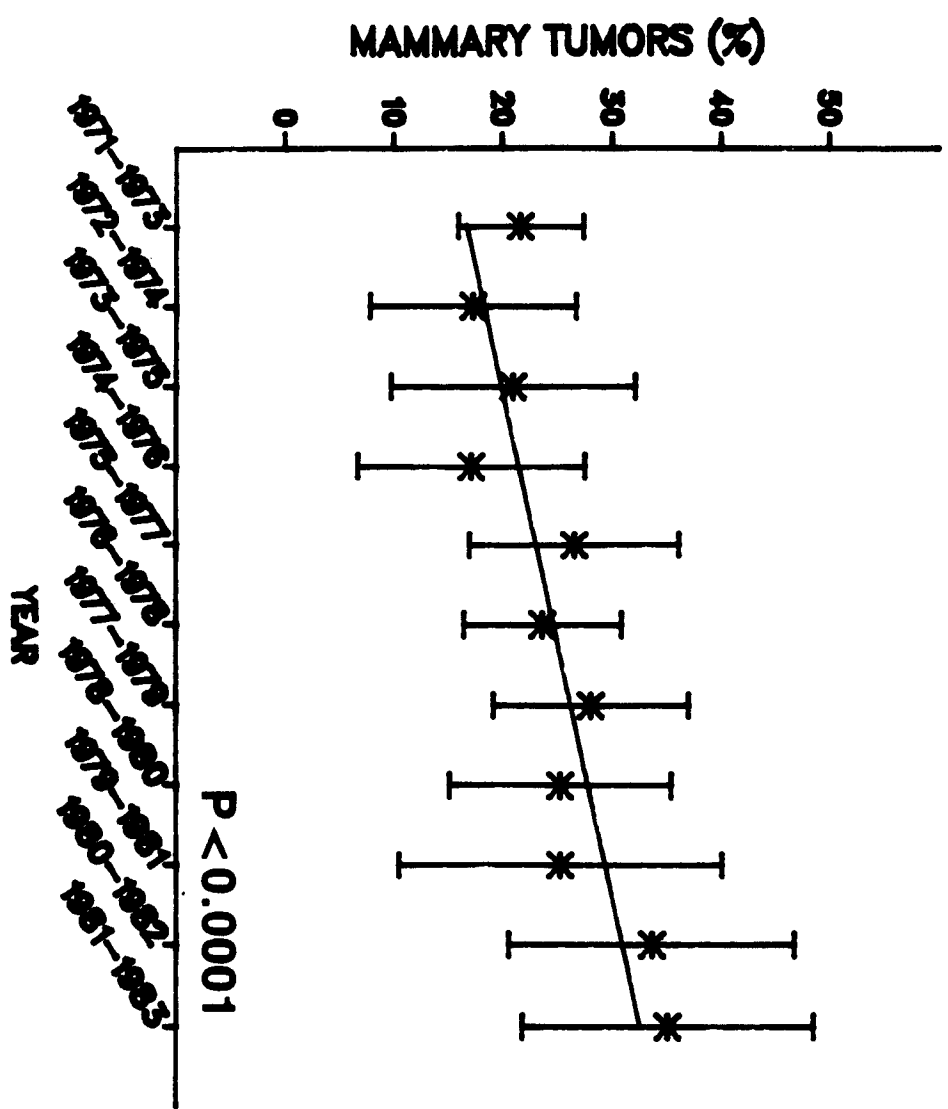
FEMALE RATS

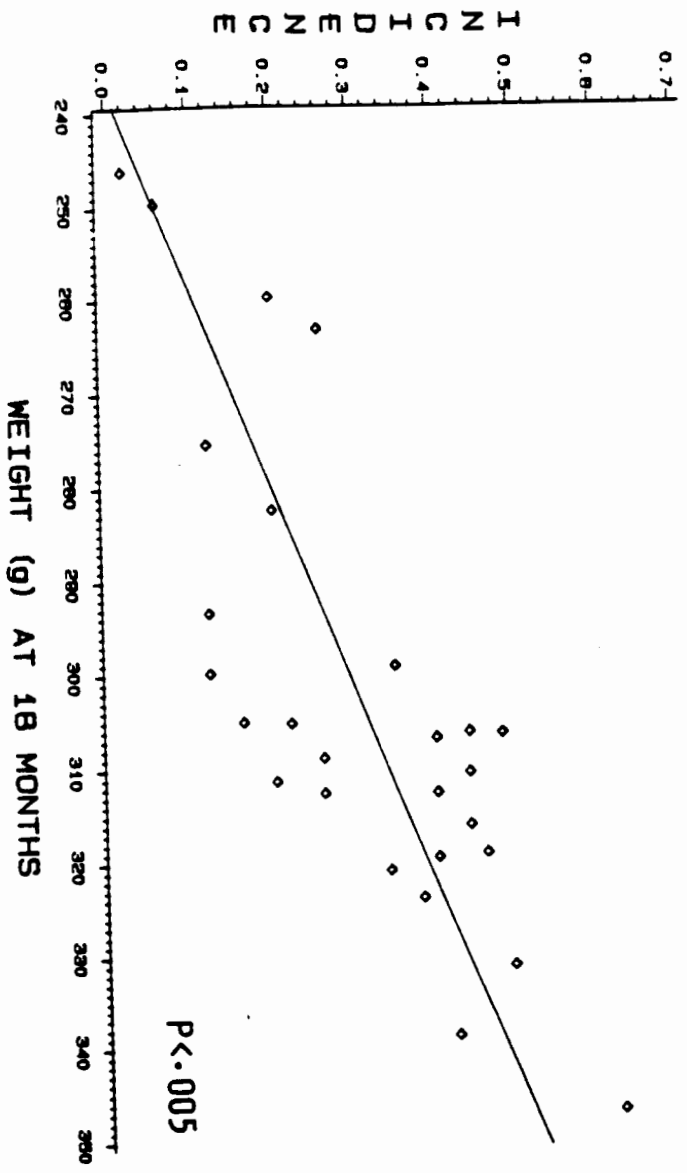


FEMALE RATS



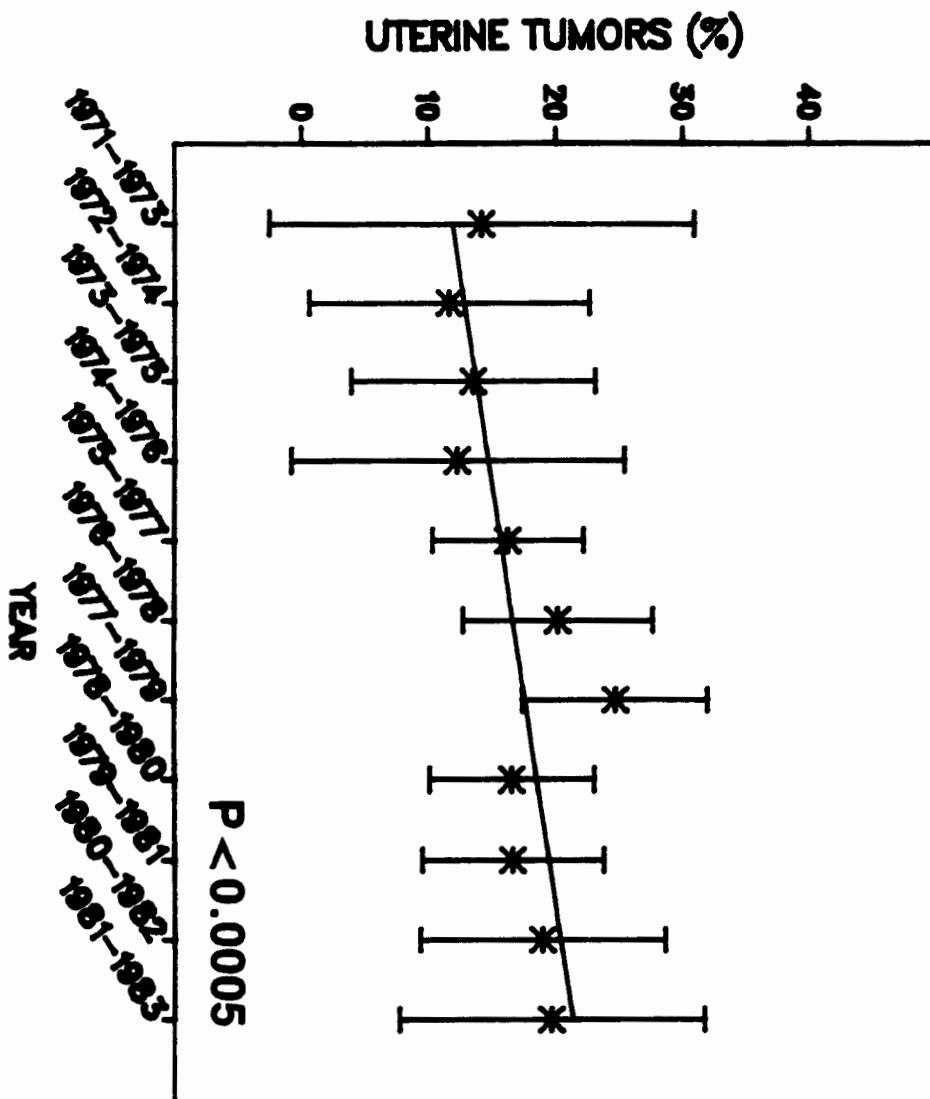
FEMALE RATS

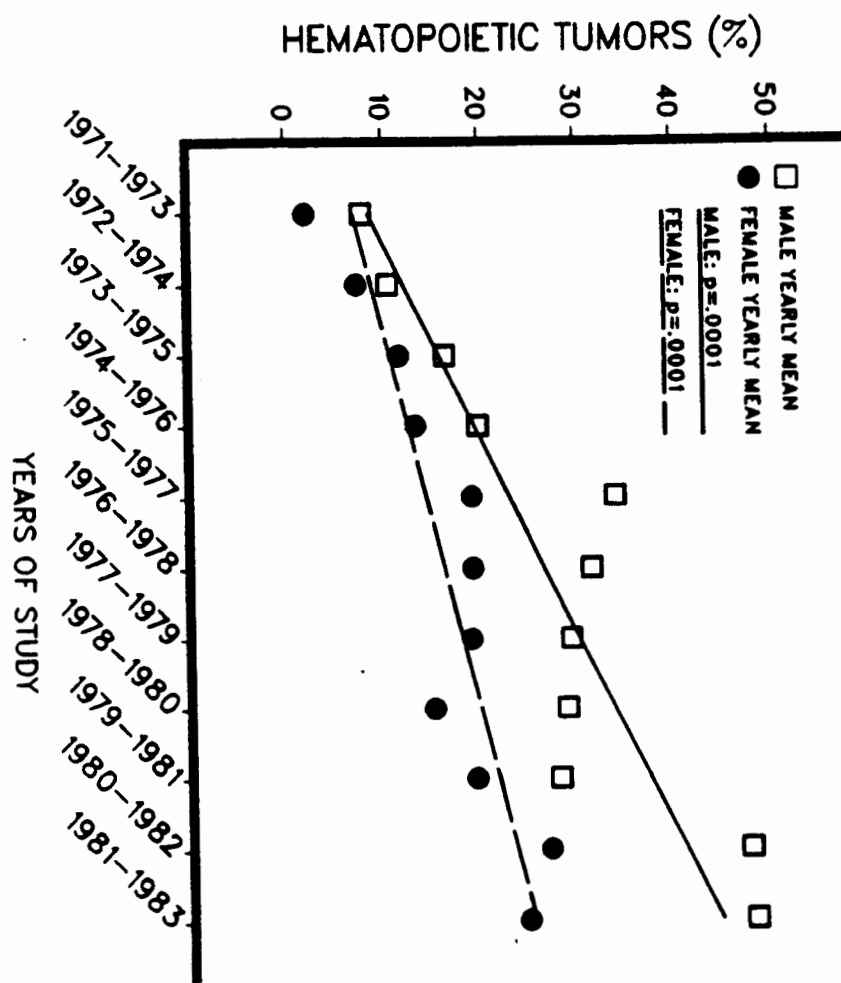




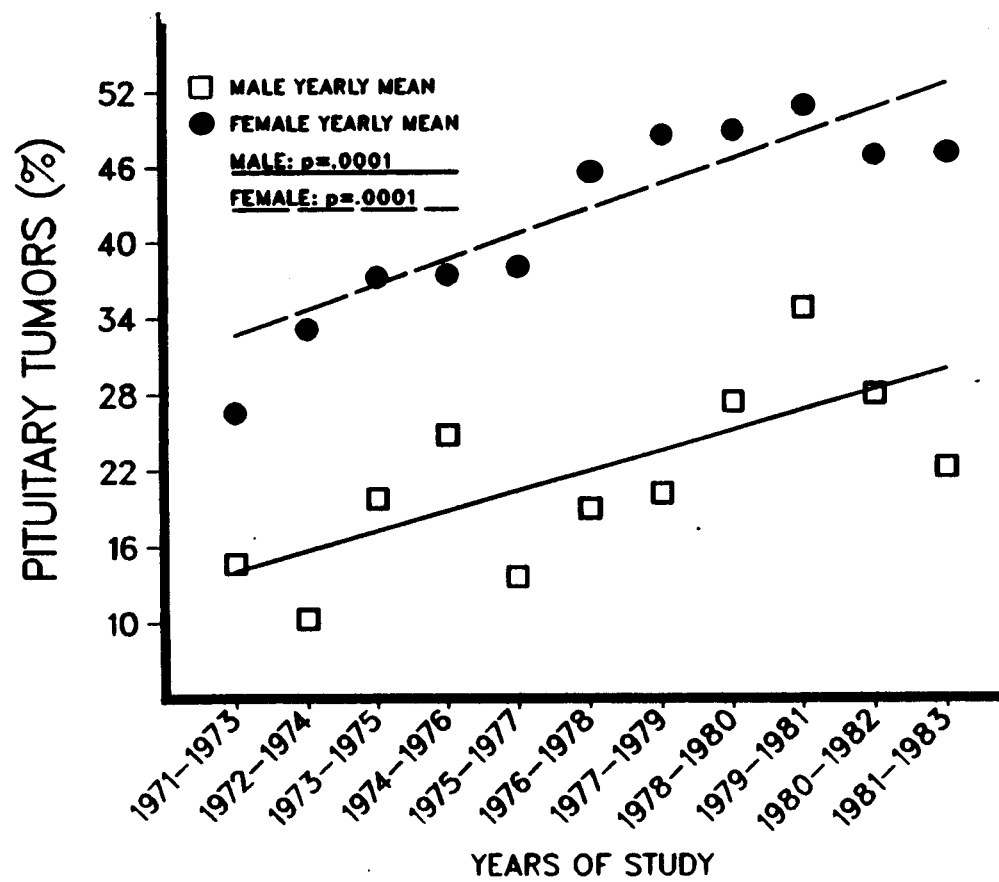
Rao et al. Am J Clin Nutr 1987; 45:252-60

FEMALE RATS





24



MICE

<u>YEAR</u>	<u>NUMBER OF STUDIES</u>	<u>LABORATORY</u>	<u>NUMBER OF STUDIES</u>
1973	35	BC	11
1974	15	DW	2
1975	4	FC	22
1976	10	GS	9
1977	16	HZ	3
1978	7	LB	19
1979	10	MA	21
1980	13	PR	8
1981	11	SO	20
		SR	3
		TR	3

MALE MICE

GROWTH CURVES

BODY WEIGHT

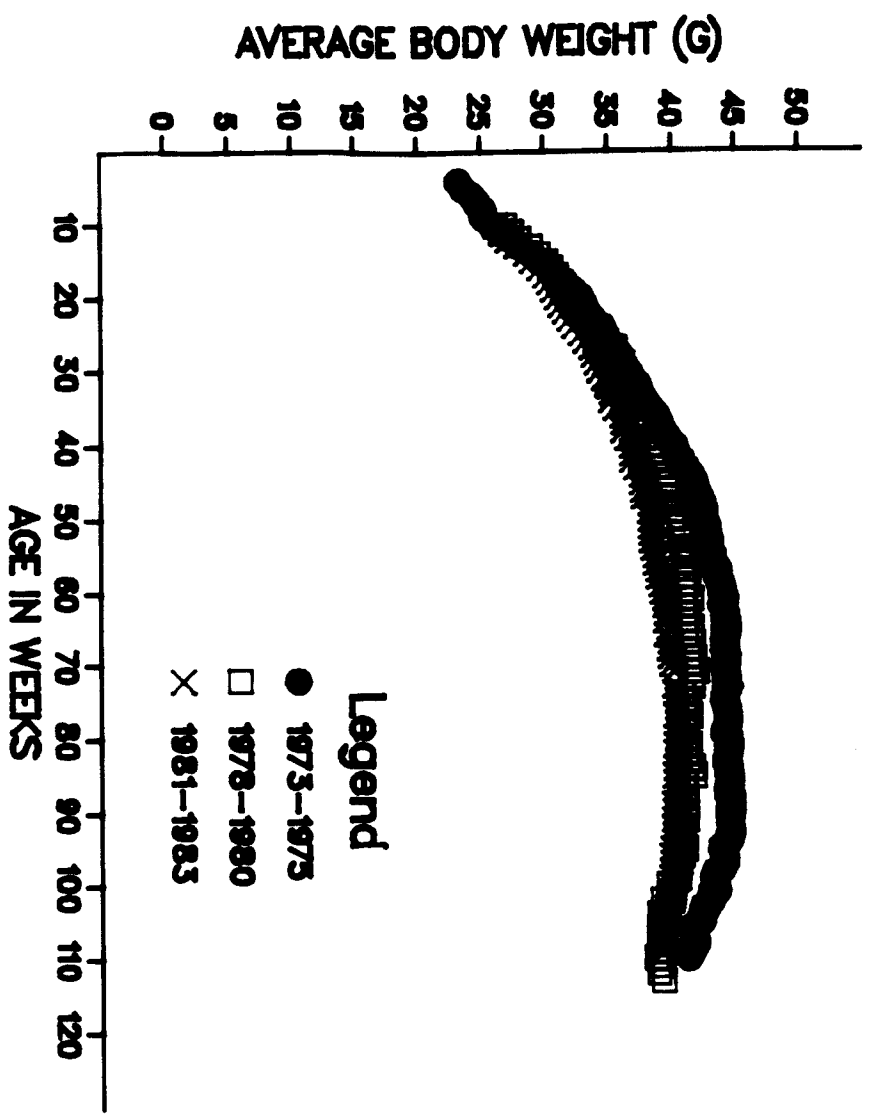
SURVIVAL

LIVER TUMORS

LUNG TUMORS

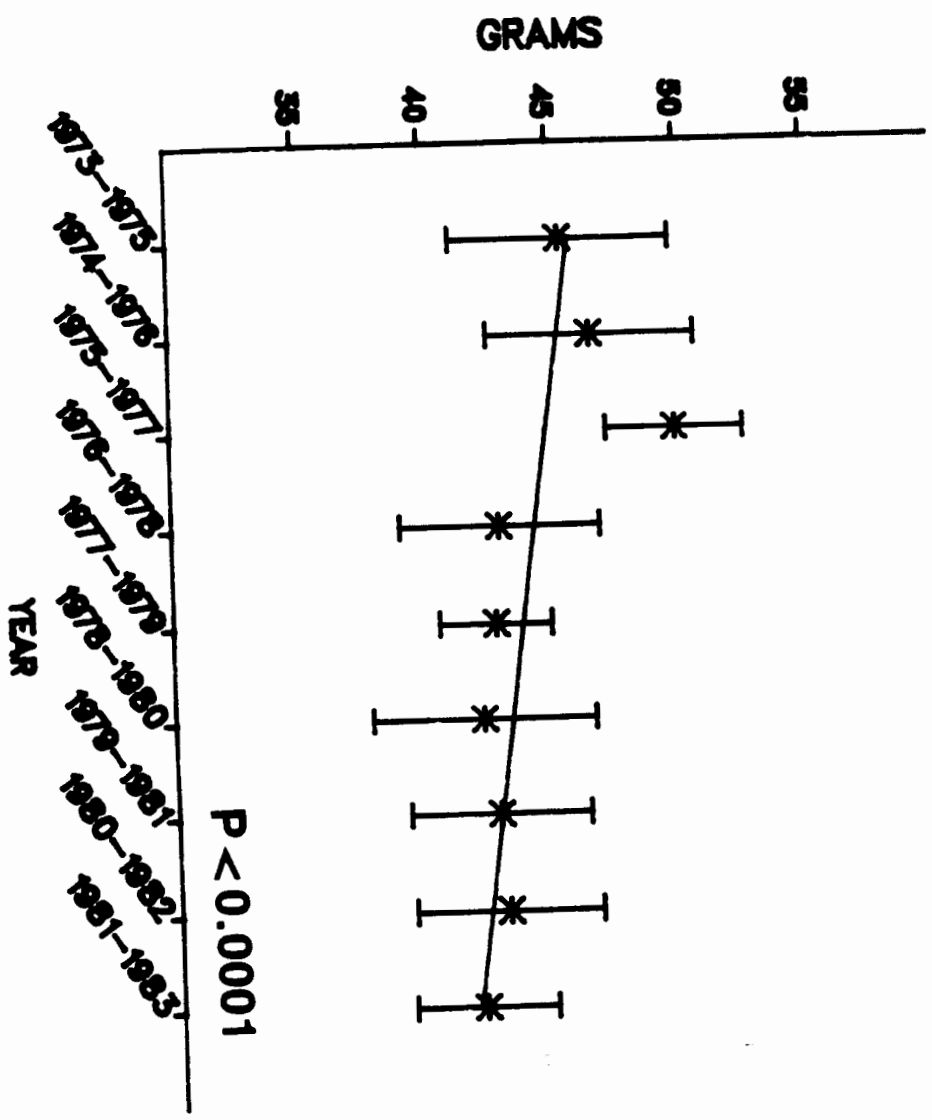
HEMATOPOIETIC SYSTEM TUMORS

MALE MICE

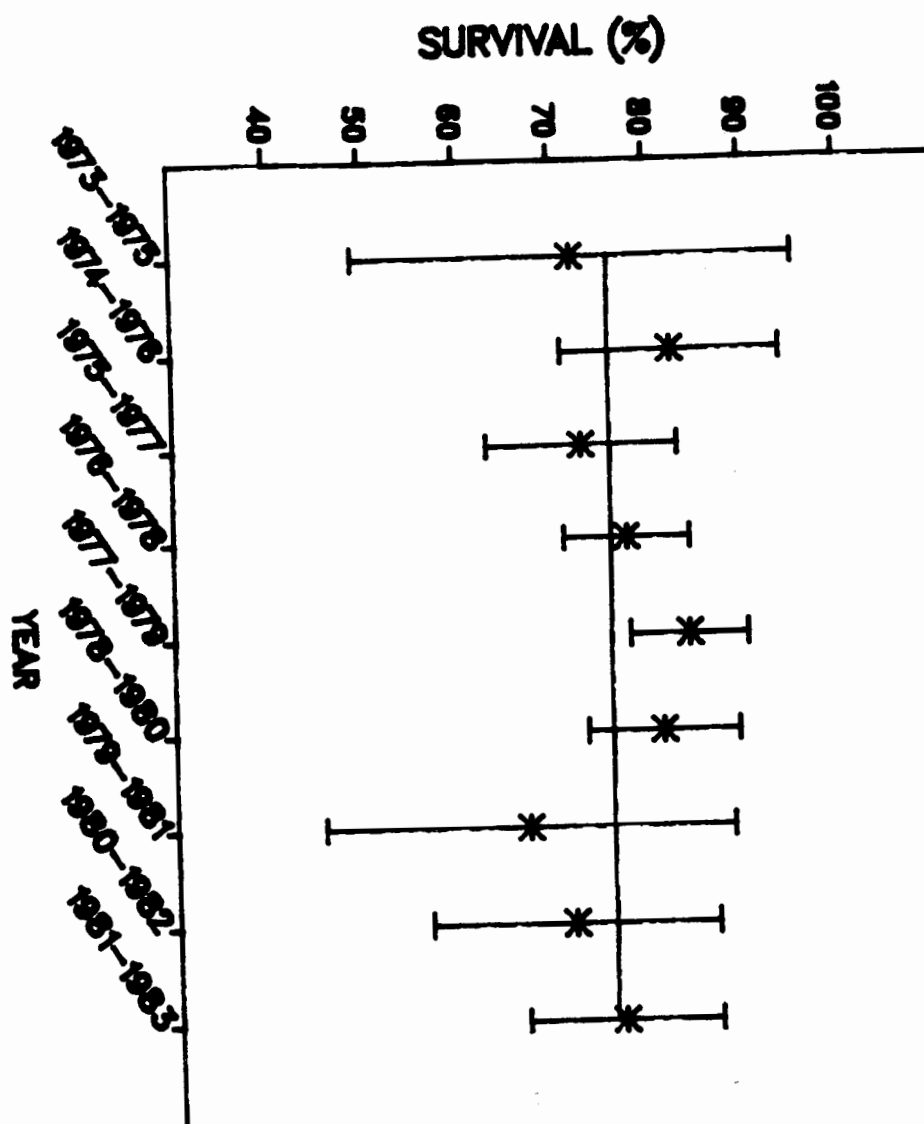


MALE MICE

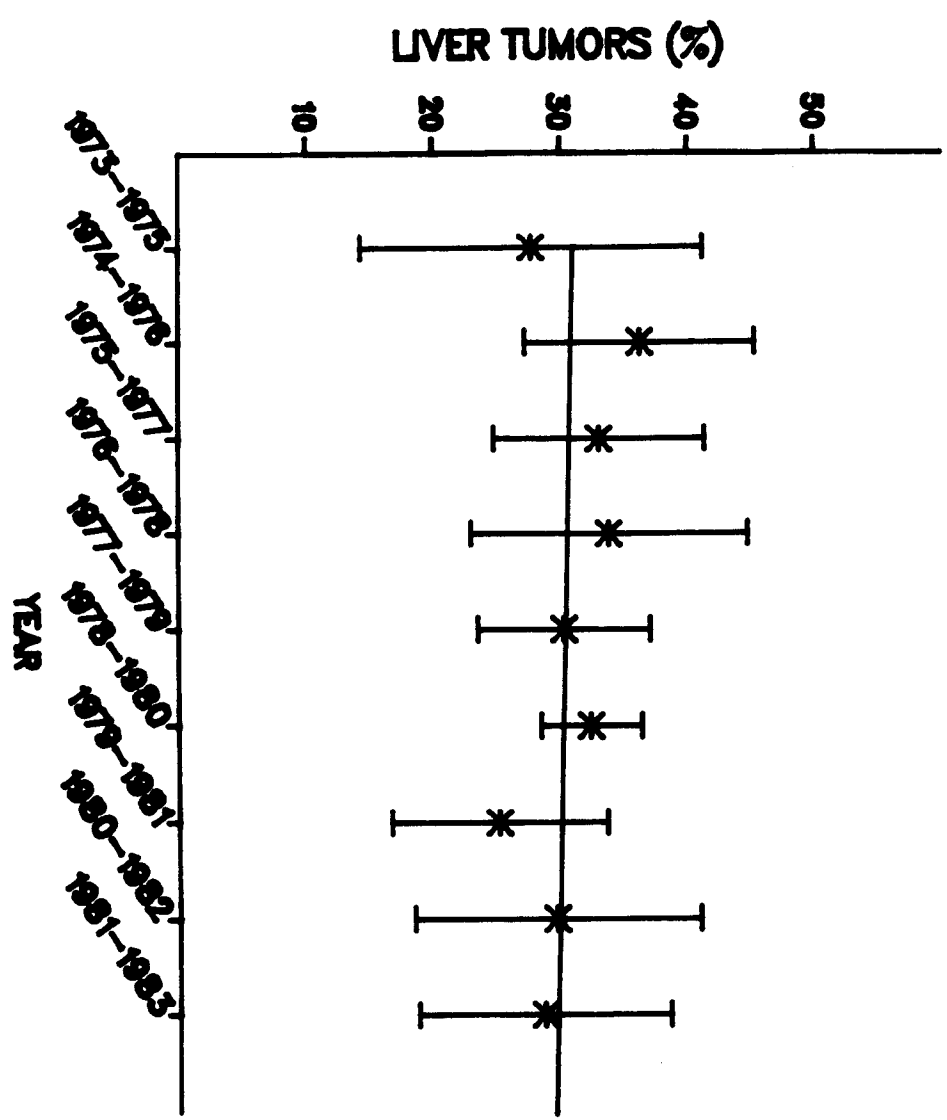
MAXIMUM MEAN BODY WEIGHT



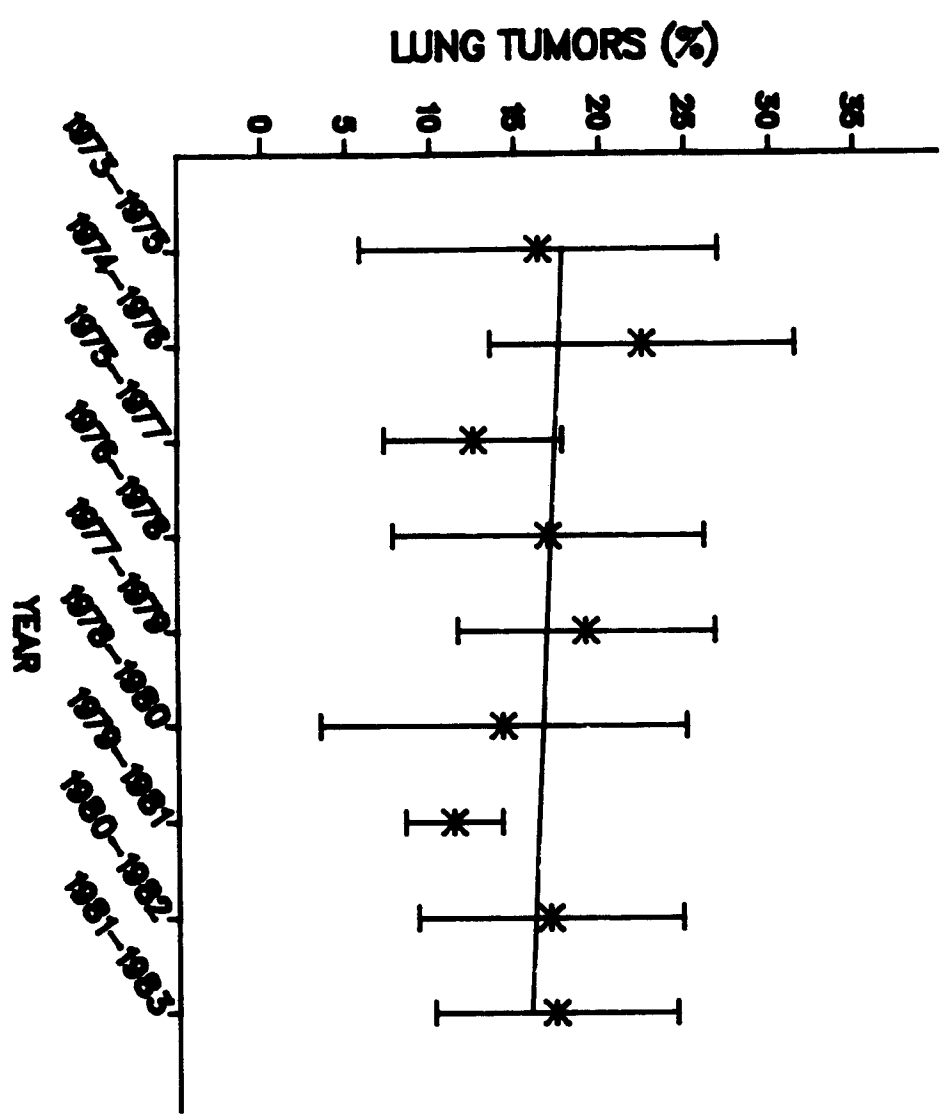
MALE MICE

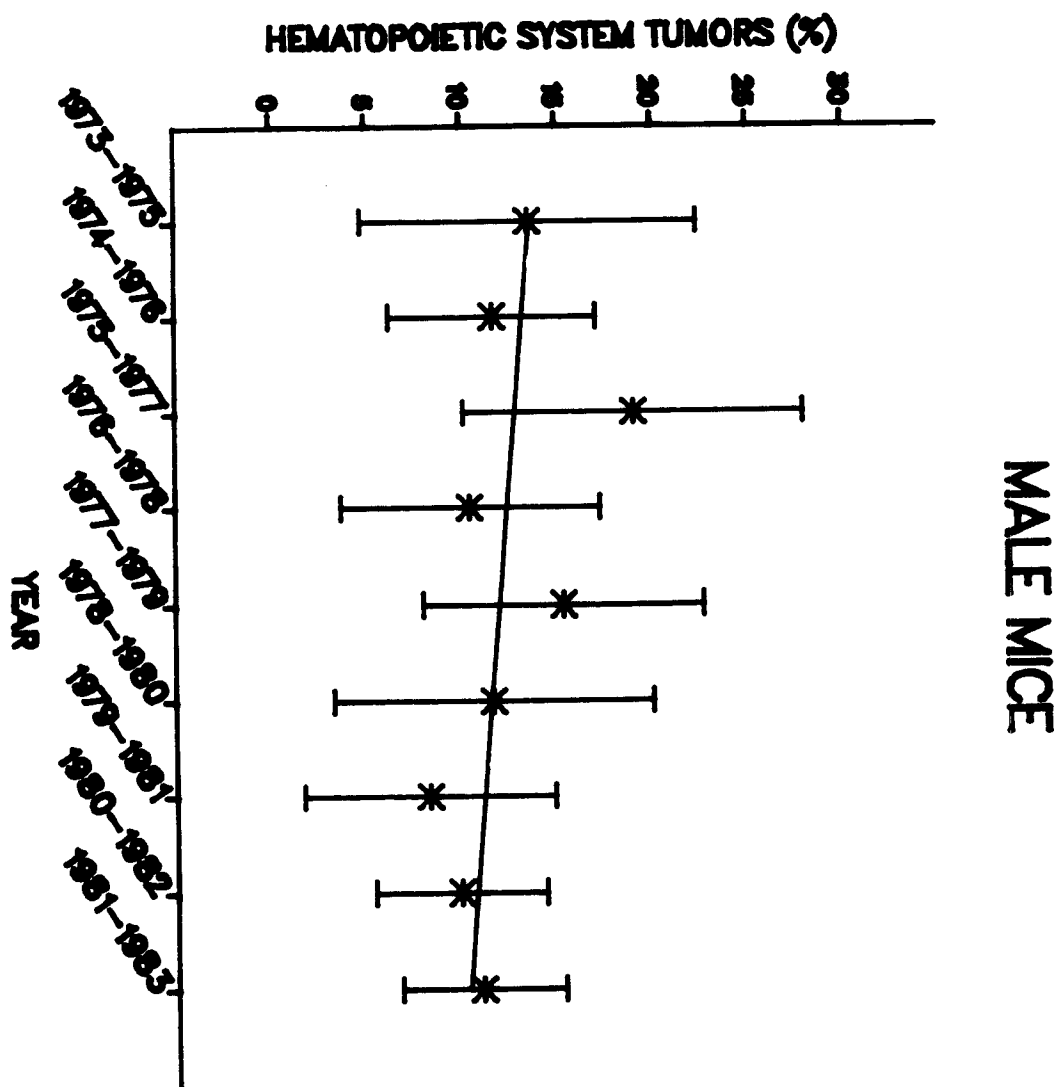


MALE MICE



MALE MICE





FEMALE MICE

GROWTH CURVES

BODY WEIGHT

SURVIVAL

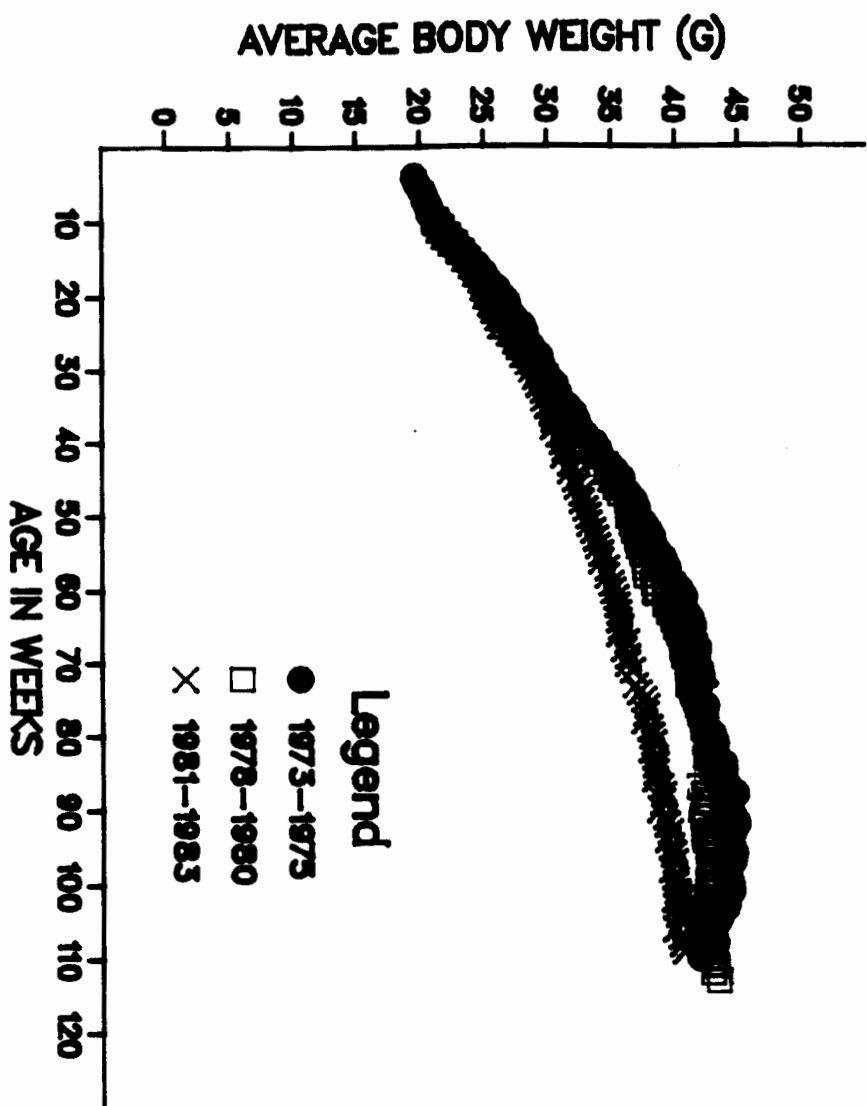
LIVER TUMORS

LUNG TUMORS

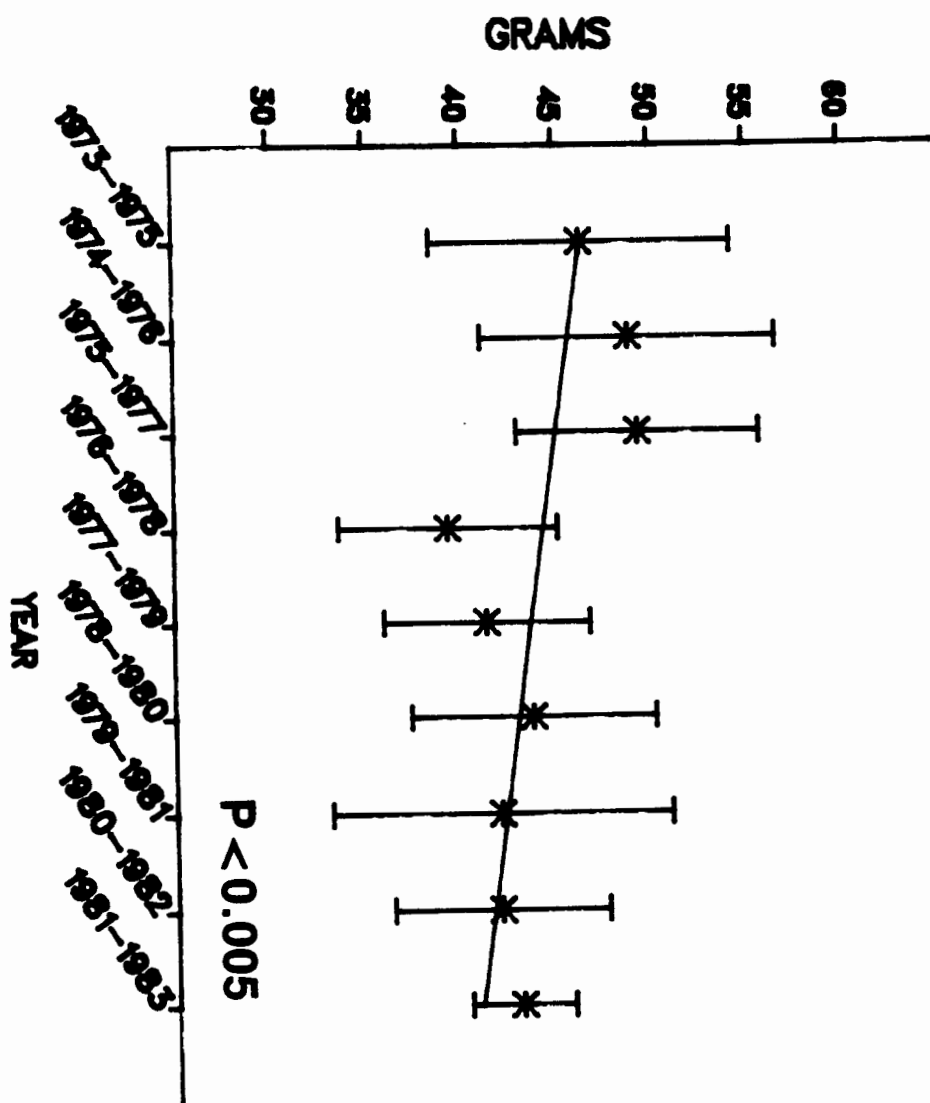
HEMATOPOIETIC SYSTEM TUMORS

ANTERIOR PITUITARY TUMORS

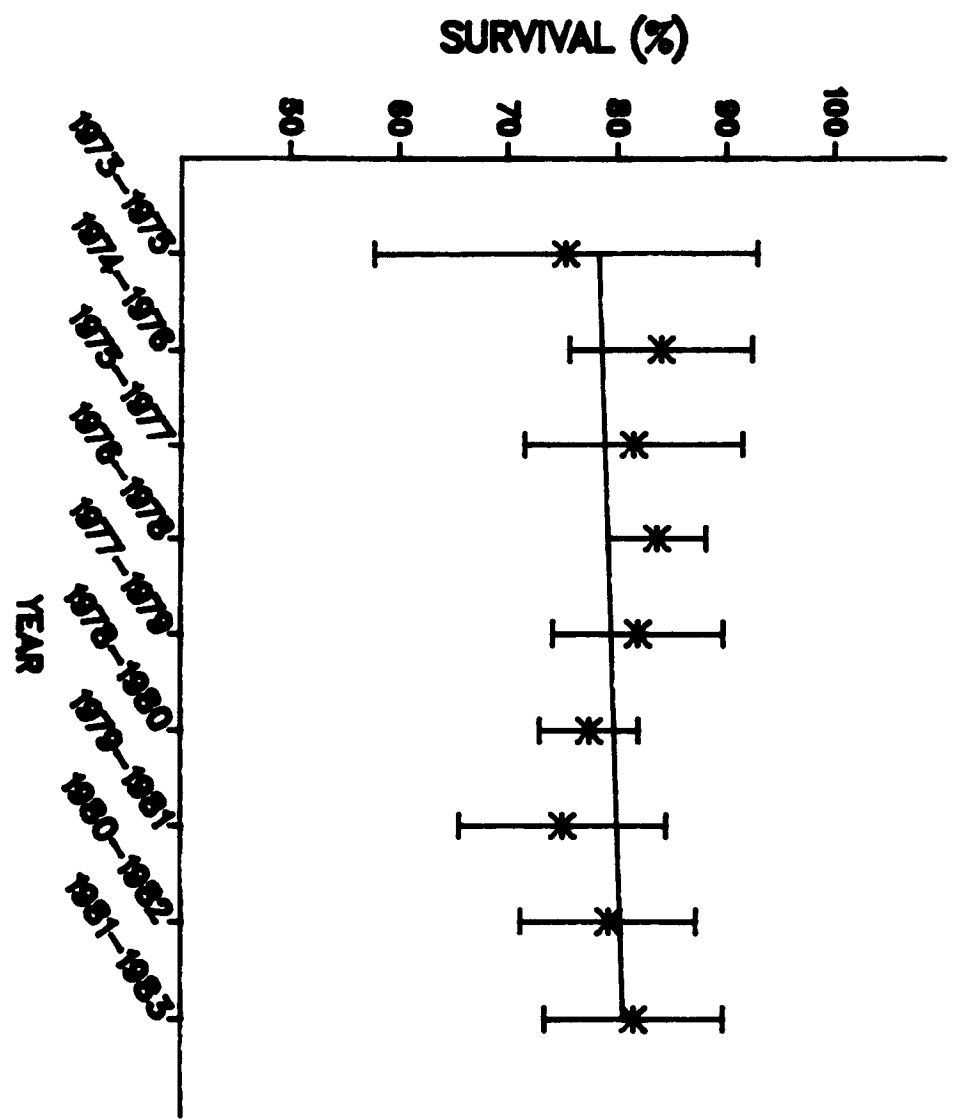
FEMALE MICE



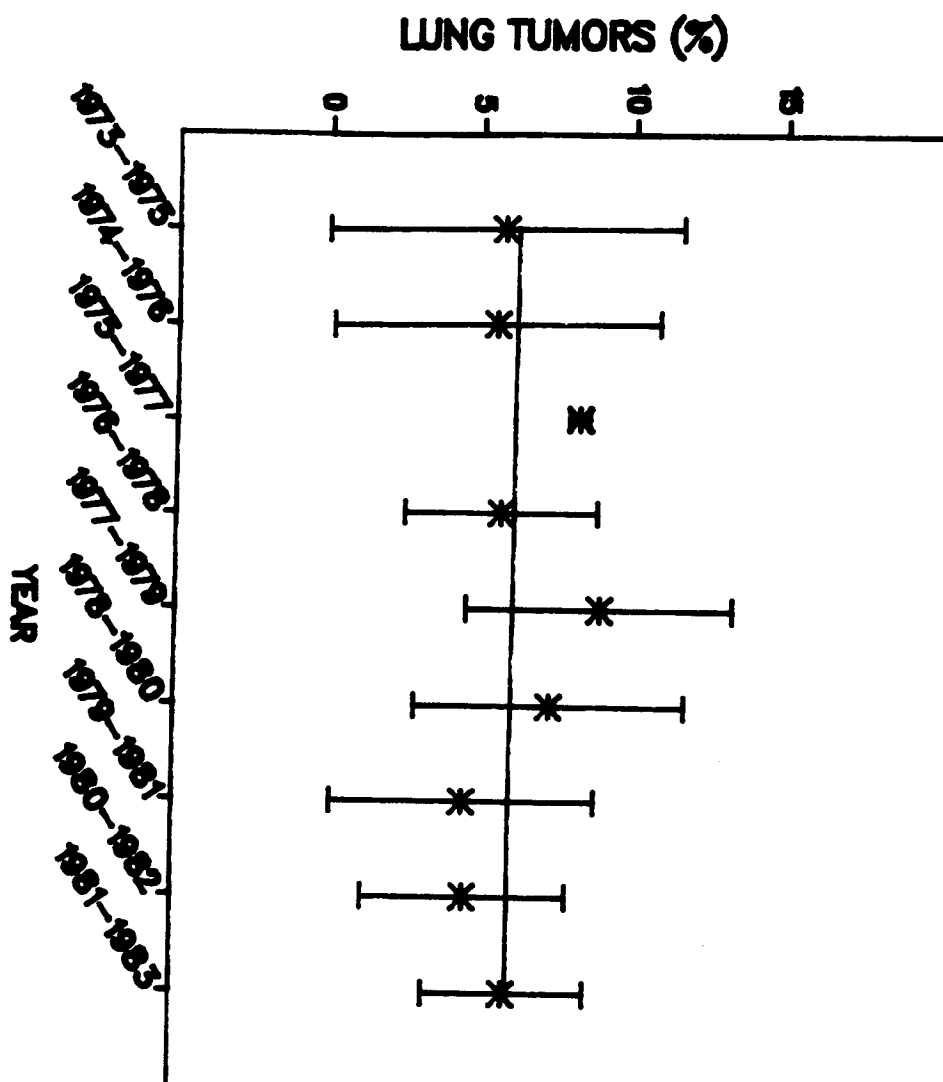
FEMALE MICE
MAXIMUM MEAN BODY WEIGHT



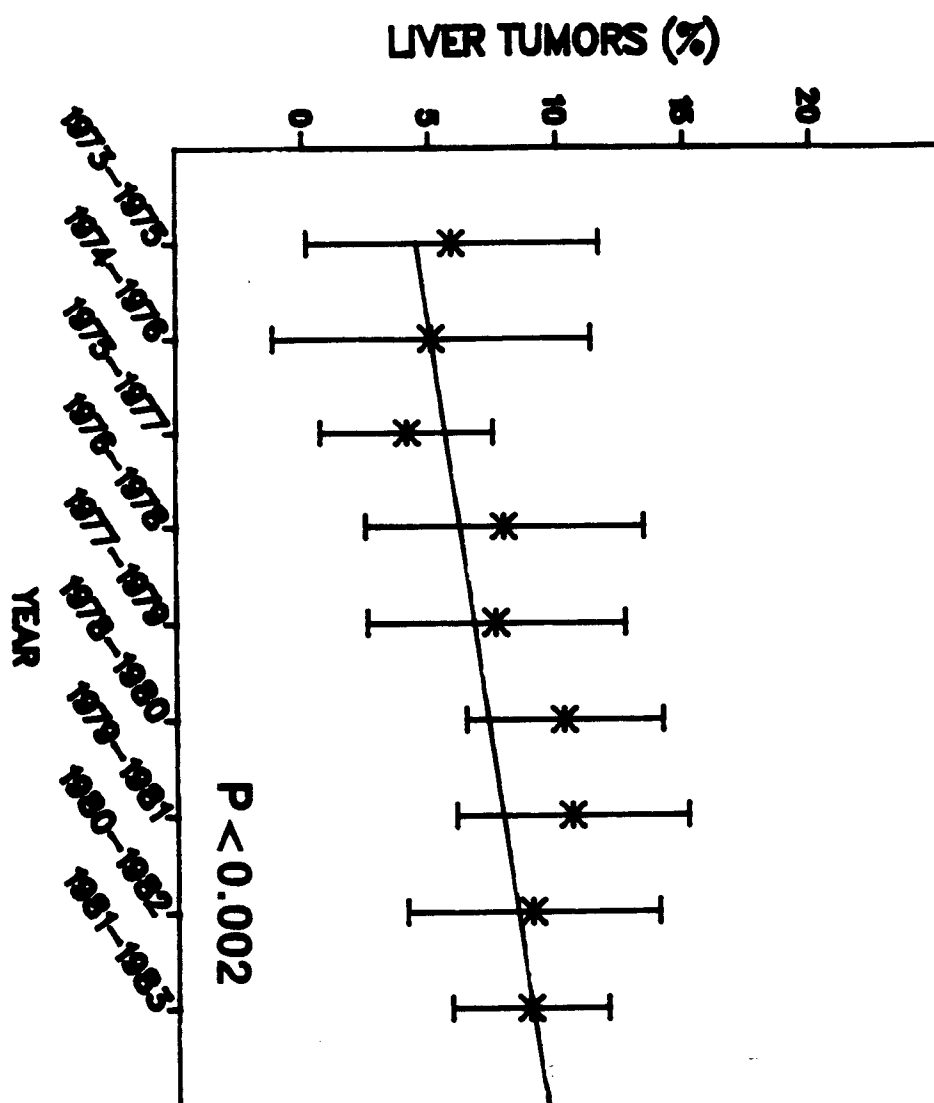
FEMALE MICE



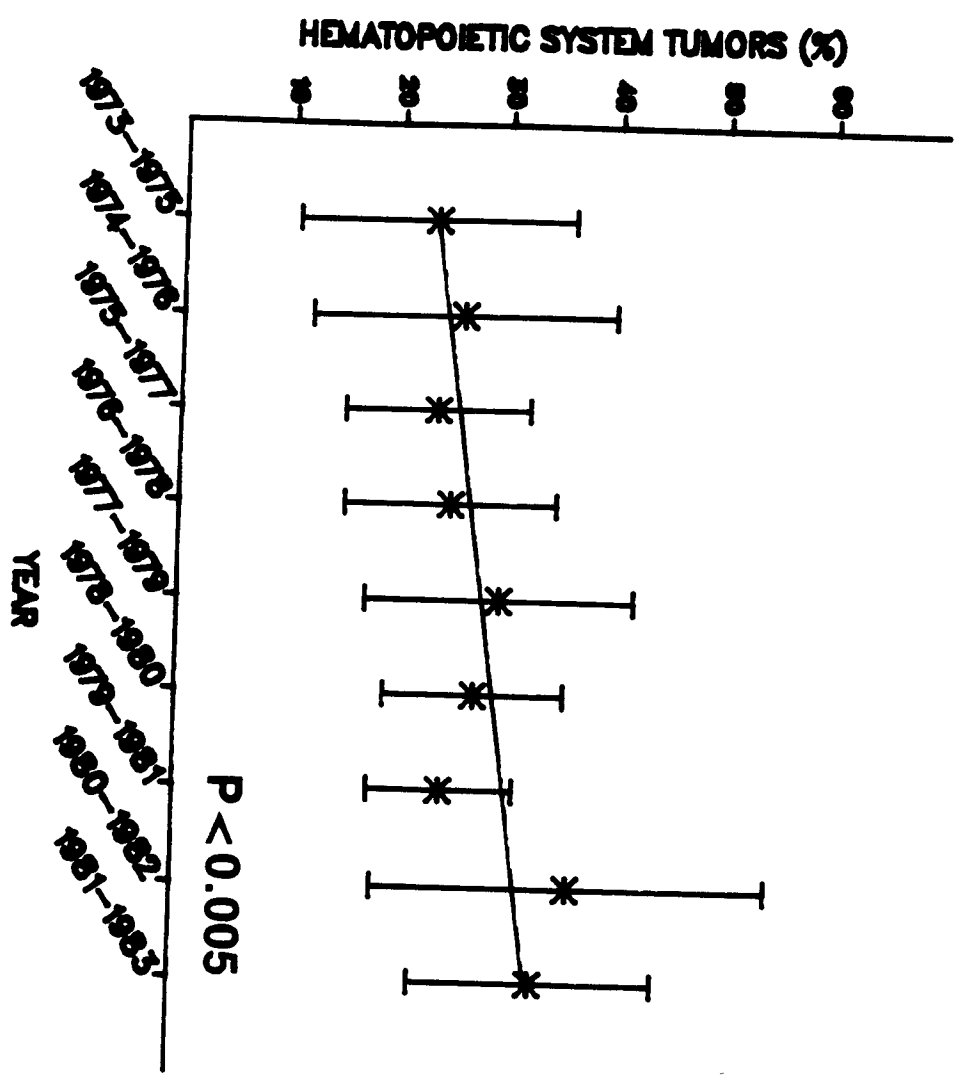
FEMALE MICE



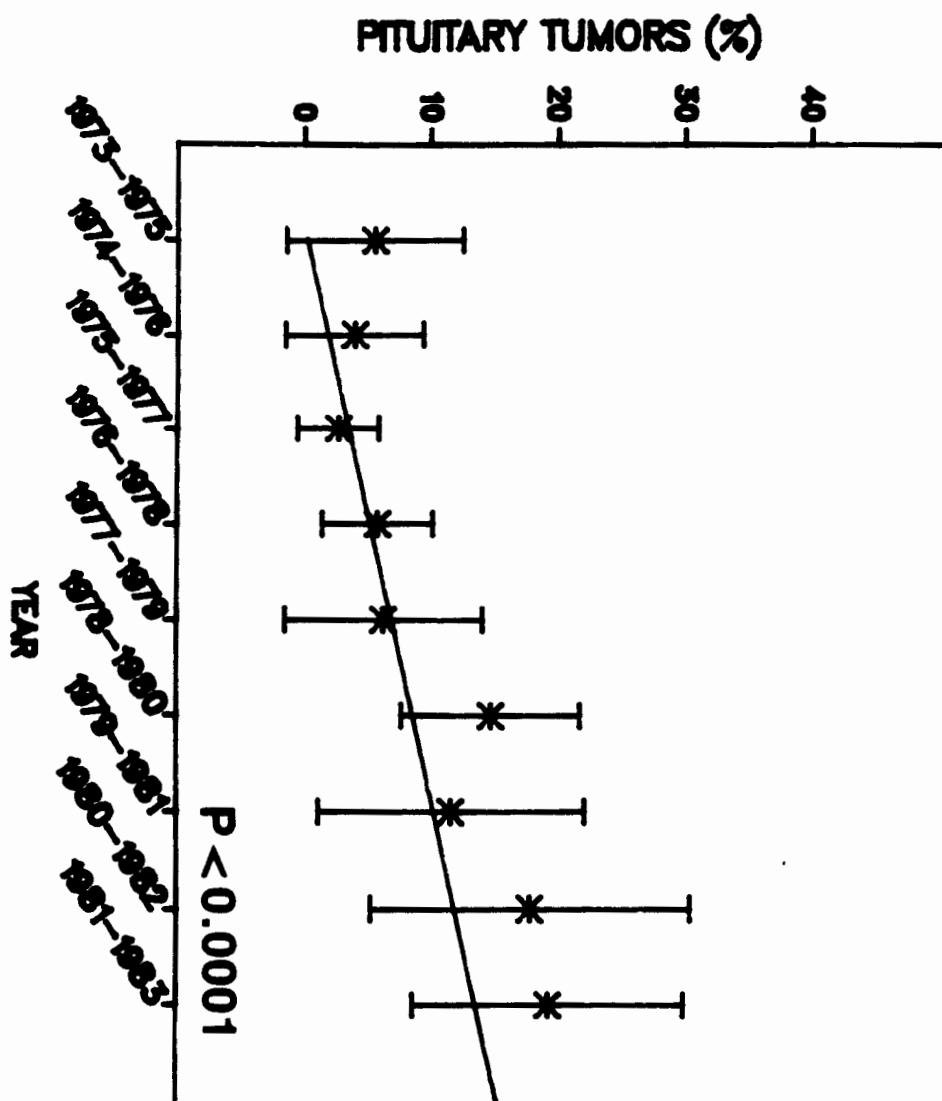
FEMALE MICE



FEMALE MICE



FEMALE MICE



41

	<u>MALE RATS</u> P Value*	<u>FEMALE RATS</u> P Value*
BODY WEIGHT	0.0001 ↑	0.0001 ↑
SURVIVAL	0.0001 ↓	NS
LEUKEMIA	0.0001 ↑	0.0001 ↑
PITUITARY	0.0001 ↑	0.0001 ↑
THYROID	0.0003 ↑	0.0001 ↑
ADRENAL	0.0001 ↑	—
MAMMARY	—	0.0001 ↑
UTERUS	—	0.0007 ↑

* Fit of the regression line for time trends.

↑ Increase ↓ Decrease

NS P>0.05 (not significant)

— Not applicable

YEAR	LABORATORY	NUMBER OF STUDIES	YEAR	LABORATORY	NUMBER OF STUDIES
1971	LB	2	1977	BC	3
	MA	1		FC	1
				MA	7
1972	LB	9		SO	2
	MA	6	1978	BC	2
	SO	7		GS	2
1973	DW	2		LB	1
	FC	17		MA	2
	LB	12		PA	1
	MA	4		SO	4
	SO	7	1979	FC	1
1974	FC	4		GS	5
	LB	4		MA	3
	MA	2		SO	2
1975	HZ	1	1980	GS	2
	SO	1		HZ	2
1976	BC	1		PR	5
	HZ	1		SO	1
	LB	4	1981	BC	2
	MA	2		PR	3
	SO	1		SR	3

DIET

CONCLUSIONS OF THE DISCUSSION SESSION

Purified diet may be the proper diet for rodents in chemical carcinogenicity studies. Data base should be developed by comparing natural ingredient and purified diets with known chemicals. Continue using natural ingredient diet until the data base is developed.

The protein content of the purified or natural ingredient diet should be 15 to 18% for growth and 8 to 12% for maintenance. Practicability and advantages of using the low protein maintenance diet for adult rodents should be evaluated in studies with selected chemicals.

Not much could be done with caloric density of the rodent diet for testing unknown chemicals. Diet restriction or pair feeding procedures are labor intensive and will require daily feeding and individual housing of all animals. These procedures may not be practical for the two year carcinogenicity studies.

45

PURIFIED DIETS

NO KNOWN CONTAMINANTS

NO KNOWN ENZYME INDUCERS

REPRODUCIBLE NUTRIENT CONCENTRATIONS

5 TO 10 TIMES MORE COST

NOT STABLE AT ROOM TEMPERATURE
(Require Refrigeration)

LIMITED HISTORICAL DATA

LESIONS IN CONTROL ANIMALS

<u>NUTRIENT</u>	<u>DIET</u>	
	<u>NIH-07</u>	<u>NTP-88</u>
Protein %	24.0	14.5
Fat %	5.0	3.1
Fiber %	3.5	5.5
Vitamin D IU/Kg	4,000	1,000
Vitamin B12 ppb	26	50
Calcium %	1.30	0.87
Phosphorous %	1.00	0.70

EXPECTED CHANGES

- SIGNIFICANTLY LOWER BODY WEIGHT IN RATS
- SIGNIFICANTLY LOWER INCIDENCE OF ENDOCRINE TUMORS
- INCREASE IN MEAN LIFESPAN
- LOWER MAXIMUM TOLERATED DOSE

EVALUATION OF NEW DIET

- SELECT FIVE CHEMICALS TO BE ADMINISTERED BY
DIET OR WATER
- THIRTEEN WEEK STUDIES WITH ONE CONTROL AND AT LEAST
TWO DOSES OF EACH SEX AND SPECIES
- TWO YEAR STUDIES WITH ONE CONTROL AND AT LEAST ONE
(HIGH) DOSE OF EACH SEX AND SPECIES
- FOUR YEARS AND APPROXIMATELY \$500,000/YEAR
- REPORT(S) IN FY 1992

Testing Recommendations for Chemicals Reviewed by Board of Scientific Counselors
on December 15, 1987

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/ Remarks
A. Chemicals Reviewed by the Chemical Evaluation Committee on July 29, 1987.			
1. 1,4-Butanediol (110-63-4)	NCI	-Subchronic studies -Neurotoxicity -Genotoxicity -Teratology screen (Moderate)	-High production -Worker exposure -Lack of toxicological data
2. Diethylene glycol (111-46-6)	NCI	-Carcinogenicity -Chemical disposition and metabolism -Genotoxicity -Teratology screen (Moderate)	-High production -Lack of carcinogenicity data -Structural interest
3. Dipropylene glycol (25265-71-8)	NCI	-Subchronic studies including emphasis on hematological effects -Carcinogenicity -Metabolism -Mutagenicity -Teratology screen (Moderate)	-High production -Potential for human exposure -Limited toxicology data -Structural interest
4. Carbon disulfide (75-15-0)	NIEHS	-Carcinogenesis and toxicology studies -Cardiovascular toxicity -Male and female repro- ductive toxicity in 90 day studies (High)	-High production -Extensive use -High potential for exposure -Lack of chronic toxicological data

Testing Recommendations for Chemicals Reviewed by Board of Scientific Counselors
on December 15, 1987

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/ Remarks
5. Methylene- diphenyl diisocyanate (101-68-8)	United Brotherhood of Carpenters and Joiners of America	-Metabolic studies to determine extent of conversion to methylene dianiline (MDA) (High)	-Human exposure -Potential for increased usage as replacement for toluene diisocyanate, a known animal carcinogen -MDA is a known animal carcinogen -Decision for long-term toxicological studies dependent on results of metabolic studies -Route of exposure should be same as for workers
6. Oxymetholone (434-07-1)	1. NIEHS 2. NCI	-Subchronic studies -Carcinogenicity -Genotoxicity -Immunotoxicity (Moderate)	-Lack of animal toxicological data -Limited evidence of carcinogenicity in humans -IARC recommended carcino- genicity testing with high priority -Testing subject to demonstration of exposure to the chemical

Testing Recommendations for Chemicals Reviewed by Board of Scientific Counselors
on December 15, 1987

Chemical (CAS Number)	Nomination Source	Testing Recommendations (Priority)	Rationale/ Remarks
B. Chemicals Reviewed by the Chemical Evaluation Committee on September 29, 1987.			
1. Heptachlor (76-44-8)	Dr. D.R. Mattison	-Perinatal toxicity studies (Low)	-Potential for exposure -Contaminant of milk and other dairy products and meats in Arkansas, Missouri and Oklahoma -Include in class study of lipid soluble com- pounds that persist in the body and are trans- ferred neonatally through lactation.
2. Heptachlor epoxide (1024-57-3)			
3. Ozone (10028-15-6)	a) Dept. of Health Services, State of California b) Health Effects Institute	-Carcinogenicity -Genotoxicity (High)	-Widespread exposure to general population -High chemical reactivity -Lack of adequate carcinogenicity data -Scientific data suggesting genotoxic and carcinogenic effects -Consider conducting co- carcinogenicity studies with carcinogenesis studies -Consider need for additional reproductive toxicity studies
4. Primaclone (125-33-7)	NCI	-Carcinogenicity (High)	-High production and human exposure -IARC recommended carcino- genicity testing with a high priority -Structurally related to tumor promotor phenobarbital