Board of Scientific Counselors
National Toxicology Program

Summary Minutes
from

Peer Review of Draft Technical Reports of Long-Term
Toxicology and Carcinogenesis Studies and Short-Term Toxicity Studies
by the Technical Reports Review Subcommittee
and Panel of Experts

on

November 19-20, 1990

Research Triangle Park, North Carolina

The review meeting began at 9:00 a.m. on November 19 and 20 in the Conference Center, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina. Members of the Subcommittee are: Drs. Robert Scala (Chairperson), Paul Bailey, Jay Goodman, Daniel Longnecker, and Ellen Silbergeld. Members of the Panel of Experts are: Drs. John Ashby, Gary Carlson, Harold Davis, Robert Garman, Lois Gold, David Hayden, Curtis Klaassen, Barbara McKnight and Lauren Zeise. These minutes have been reviewed and approved by all members of the Subcommittee and Panel. They were written by Dr. Larry G. Hart, Executive Secretary.

When available, a limited number of final NTP Technical Reports for the studies may be obtained free of charge from the NTP Public Information Office, MD B2-04, P. O. Box 12233, Research Triangle Park, NC 27709. Telephone: (919) 541-3991; FTS: 629-3991. Subsequently, they may be purchased from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, Virginia 22161, (703) 487-4650.

The next NTP technical reports peer review meeting will be held March 11-12, 1991, in Research Triangle Park, North Carolina. For information, contact Dr. Hart, (919) 541-3971; FTS 629-3971.

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SUMMARY MINUTES

PEER REVIEW PANEL MEETING

November 19-20, 1990

Acetaminophen. Dr. R. Irwin, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of acetaminophen by discussing uses of the chemical, reporting on the experimental design for the studies, and reviewing the nonneoplastic and neoplastic lesions in male and female rats and mice. The proposed conclusions were that:

> Under the conditions of these 2-year feed studies, there was no evidence of carcinogenic activity of acetaminophen in male F344/N rats that received diets containing 0, 600, 3,000, or 6,000 ppm acetaminophen. There was some evidence of carcinogenic activity of acetaminophen in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was no evidence of carcinogenic activity in male and female B6C3F1 mice that received diets containing 0, 600, 3,000, or 6,000 ppm acetaminophen. Nonneoplastic lesions associated with exposure to acetaminophen included increased severity of nephropathy and increased incidences of parathyroid hyperplasia in male rats, increased severity of nephropathy in female rats, and increased incidences of thyroid follicular cell hyperplasia in male and female mice.

Dr. Garman, a principal reviewer, agreed with the conclusions for male rats and male and female mice but had reservations about the conclusions for female rats. He asked for information on the historical control frequency of multiple organ involvement for mononuclear cell leukemias in female rats. Dr. Irwin said there was not a historical control data base pertaining to degree of multiple organ involvement. Dr. S. Eustis, NIEHS, commented that in female rats which have lower spontaneous incidence than males, a less extensive degree of organ involvement is typical, with spleen and liver being the organs primarily affected.

Dr. Goodman, the second principal reviewer, agreed with the conclusions. However, he suggested adding a dependent clause after the conclusion for female rats as follows, "a malignancy which exhibits a high spontaneous rate and variable incidence in controls". Dr. Goodman also said the possibility should be considered that the sister chromatid exchanges and chromosomal aberrations reported might be artifacts resulting from lysosome breakdown secondary to cytotoxicity, as opposed to a direct action of the chemical on DNA. Dr. E. Zeiger, NIEHS, commented that the way the studies are done would not provide information on such a mechanism.

Dr. Carlson, the third principal reviewer, agreed with the conclusions. He noted the reference to the study by Sandler would suggest that epidemiology

studies have been done in humans and wondered if there were other studies which might be relevant and should be cited.

Dr. Gary M. Williams, representing McNeil Consumer Products, stated that acetaminophen played an important role in clinical medicine and that reports of neoplastic effects should be derived from sound data which he did not think was the case with the NTP study. He expressed the view that there was not an increased incidence of mononuclear cell leukemia in males, there was no dose-response on shortened tumor latency in females, there was extreme variability in leukemia across control groups, and studies by others had not reported increased incidence of leukemia. Dr. J. Haseman, NIEHS, disagreed, responding that indeed there was a dose-response in female rats, there was some indication of shortened tumor latency, and the historical variability in leukemia across laboratories was no greater than that expected by chance alone.

Dr. Zeise expressed concern about the adequacy of the studies in mice, noting that survival, mean body weights and clinical findings in subchronic and chronic studies suggested mice may have tolerated a higher top dose. She questioned administration of acetaminophen by food rather than bolus dose which would be more similar to human exposure.

Dr. Garman moved that the Technical Report on acetaminophen be accepted with revisions discussed and the conclusions as written for male rats and male and female mice, no evidence of carcinogenic activity, but with the conclusion for female rats to be changed from some evidence of carcinogenic activity to equivocal evidence of carcinogenic activity. Dr. Ashby seconded the motion, supporting the change in female rats because of cumulative uncertainty regarding the significance of mononuclear cell leukemia as to being treatment-related rather than chemically-related. Dr. Gold noted that in a 30-month study in Japan using F344 rats, the same strain as in the NTP studies, an increased incidence of mononuclear cell leukemia was not observed. Dr. McKnight opined that with a variable tumor such as leukemia, the concurrent control should be given most weight. Dr. Zeise offered an amendment that a statement be added to the conclusion "that mice might have tolerated higher doses". Dr. Carlson seconded the amendment which was defeated by nine no to two yes votes (Carlson, Zeise) with one abstention (McKnight). The main motion was then accepted by eight yes votes (Ashby, Davis. Garman, Gold, Hayden, Klaassen, Longnecker, Zeise) to four no votes (Carlson, Goodman, McKnight, Silbergeld).

Chlorinated and Chloraminated Water. Dr. J.K. Dunnick, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of chlorinated and chloraminated water by discussing uses, experimental design, survival and water consumption in rats and mice, and results. The proposed conclusions were that:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of chlorinated drinking water for male F344/N rats. There was equivocal evidence of carcinogenic activity of chlorinated drinking water for female F344/N rats, as indicated by an increase in the incidence of mononuclear cell leukemia. There was no evidence of carcinogenic activity of chlorinated drinking water for male or female B6C3F1 mice.
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> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of chloraminated drinking water for male F344/N rats. There was equivocal evidence of carcinogenic activity of chloraminated drinking water for female F344/N rats, as indicated by an increase in the incidence of mononuclear cell leukemia. There was no evidence of carcinogenic activity of chloraminated drinking water for male or female B6C3F1 mice.

Dr. Longnecker, a principal reviewer, agreed with the conclusions but asked for more discussion of the rationale for interpretation of data for male rats. He asked why there was such low survival in male rats including controls and what impact this may have had on the validity of the study. Dr. S. Eustis, NIEHS, commented that higher incidences of leukemias, pituitary gland tumors and kidney disease contributed to lower survival in male rats in this as well as other more recent studies.

Dr. Goodman, the second principal reviewer, agreed with the conclusions in male rats and male and female mice but did not agree with the conclusions on the level of evidence for leukemia in female rats which he thought should be changed to no evidence of carcinogenic activity. He based his recommendation on the high and variable incidence of leukemias in historical controls such that overall rates of leukemias in treated groups were within the historical control range, a "low" concurrent control incidence, and a lack of a dose-response relationship. Further, the statistical significance ($P < 0.05$) was marginal for such a commonly occurring neoplasm. Dr. Dunnick responded that primary emphasis is given to concurrent control values. Dr. Goodman asked that additional information be added to the Introduction section concerning the carcinogenicity of chloroform in drinking water; he provided two literature references to be cited.

Dr. Ashby, the third principal reviewer, agreed with the conclusions. He commented that the title and other references to 'chlorinated drinking water' were inaccurate because the water used in the study had been treated with activated carbon and deionized prior to chlorination. Dr. Dunnick said words would be added to the Abstract and elsewhere to point out that the studies were intended to determine toxicity and carcinogenicity of chorinated or chloraminated water without confounding effects of byproducts. Dr. Ashby expressed concern about the poor survival of male rats and how this related to adequacy of the study. Dr. J. Haseman, NIEHS, said that in the judgment of the NTP, sur-

vival of male rats was sufficient to permit an evaluation of carcinogenicity. Dr. Zeise noted that squamous cell tumors of the upper gastrointestinal tract were elevated in male rats. Dr. Eustis said they were not believed to be compound-related.

There was some discussion as to whether text and references to effects of trihalomethanes should be included in the introduction since the water purification processes used in this study would have removed or prevented formation of any of these chemicals. Dr. Silbergeld questioned relevance to human exposure. There was also considerable discussion about the variability and increasing incidence of mononuclear cell leukemias in rats and how this affected the interpretation of the findings in dosed female rats.

Dr. Longnecker moved that the draft Technical Report on chlorinated and chloraminated water be accepted with the revisions discussed including a modification of the report title to describe the use of charcoal filter deionized water. He moved that the conclusions be accepted as written, no evidence of carcinogenic activity, for male rats and male and female mice, and, equivocal evidence of carcinogenic activity for female rats. Dr. Ashby seconded the motion. Dr. Zeise offered an amendment that the studies of chlorinated water in male rats be considered an inadequate study of carcinogenic activity due to problems of poor survival and insufficient dosing. Dr. Silbergeld seconded the amendment which was defeated by ten no to two yes votes (Silbergeld, Zeise). Dr. Goodman offered an amendment that the conclusions in female rats be changed to no evidence of carcinogenic activity. Dr. Carlson seconded the amendment which was defeated by nine no to three yes votes (Carlson, Gold, Goodman). The original motion by Dr. Longnecker was then accepted by eight yes to three no votes (Carlson, Gold, Goodman) with one abstention (Silbergeld).

C.I. Direct Blue 15. Dr. J.K. Dunnick, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of C.I. Direct Blue 15 by discussing the uses, reporting on the experimental design, and reviewing the nonneoplastic and neoplastic lesions in male and female rats. The proposed conclusions were that:

> Under the conditions of these 22-month drinking water studies, there was clear evidence of carcinogenic activity of C.I. Direct Blue 15 (desalted industrial grade) for male F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, preputial gland, liver, oral cavity, and small and large intestine. Increased incidences of mononuclear cell leukemia and neoplasms of the brain may have been related to chemical administration. There was clear evidence of carcinogenic activity for female F344/N rats, as indicated by benign and malignant neoplasms of the skin, Zymbal's gland, clitoral gland, liver, oral cavity, small and large intestine, and uterus, and mononuclear cell leukemia. Increased incidences of neoplasms of the brain may have been related to chemical administration.

Dr. Dunnick explained that the studies were intended to last 24 months but were terminated after 22 months because of decreased survival of exposed animals, due primarily to neoplasia.

Dr. Klaassen, a principal reviewer, agreed with the conclusions. He questioned whether there was really an increased incidence of brain tumors in dosed female rats. Dr. Klaassen said his major concern was with the fact that the dye was only about 50 % pure and that this be indicated in the title and elsewhere.

Dr. McKnight, the second principal reviewer, did not agree with the conclusions. She said the studies should be considered inadequate unless the impurities in the mixture could be characterized and listed and the overall study entitled as being a test of industrial grade C.I. Direct Blue 15. Further, she thought the study to be relevant only if it could be documented that the impurities were typical of those to which workers were exposed. If these issues could be resolved, Dr. McKnight stated that the highly statistically significant increases in mononuclear cell leukemias in male rats supported inclusion of these lesions under clear evidence. She also noted that increased incidences of adrenal pheochromocytomas might be considered as part of the evidence in males. Dr. Dunnick responded that pheochromocytomas are commonly occurring tumors and the fact that there was no increase in hyperplasias suggested a lack of association with chemical exposure. Dr. S. Eustis, NIEHS, agreed that mononuclear cell leukemia could be considered part of the evidence in male rats.

Dr. Zeise, the third principal reviewer, agreed with the conclusions. She shared the concern of the other reviewers about the composition of the dye and the need to modify the title to reflect what was tested.

In response to concerns of the reviewers about the purity of the dye, Dr. Scala noted that the dye studied was characteristic of industrial or technical grade material used, and the C.I. nomenclature was the correct way of describing dyes. Dr. Dunnick said the dye was representative of what workers were exposed to, and a subtitle would be added to the title to more fully describe the

composition of the dye. Dr. McKnight pointed out that in the conclusions, the name of the dye was followed by, in parentheses, "desalted industrial grade". Dr. Silbergeld raised the question as to whether 3,3'-dimethoxybenzidine either formed metabolically or present as an impurity could be contributing to the neoplastic effects. Dr. Ashby agreed noting that the dimethoxy compound would be formed in vivo by reductive cleavage, and pointing out that the only positive genetic toxicology finding was in Salmonella where reductive metabolism was incorporated.

Dr. Klaassen moved that the Technical Report on C.I. Direct Blue 15 be accepted with the revisions discussed including addition of a subtitle to describe the content of the dye, and with the conclusions as written for male and female rats, clear evidence of carcinogenic activity. Dr. Goodman seconded the motion. Then, three amendments were offered and voted on. Dr. Klaassen moved that the last sentence of the conclusions for female rats be deleted, i.e., "Increased incidences of neoplasms of the brain may have been related to chemical administration". Dr. Goodman seconded the motion which was accepted by nine yes to two no votes (Garman, Hayden) with one abstention (Ashby). Dr. McKnight moved that mononuclear cell leukemias be added under clear evidence in male rats. Dr. Zeise seconded the motion which was defeated by seven no votes (Ashby, Carlson, Gold, Goodman, Hayden, Klaassen, Chairman Scala breaking the tie) to six yes votes (Davis, Garman, Longnecker, McKnight, Silbergeld, Zeise). Dr. McKnight moved that adrenal pheochromocytomas be included in the conclusions for male rats as "may have been related to chemical administration". Dr. Silbergeld seconded the motion which was defeated by nine no votes (Ashby, Carlson, Davis, Garman, Goodman, Gold, Hayden, Klaassen, Silbergeld) to three yes votes (Longnecker, McKnight, Zeise). The Panel then accepted the original motion unanimously with twelve votes.

Methyl Bromide. Dr. R.S.H. Yang, NTP Staff Scientist (now at Colorado State University), and Dr. S.L. Eustis, NIEHS, were present. Dr. Eustis introduced the toxicology and carcinogenesis studies of methyl bromide by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic lesions in male and female mice. The conclusions were that:

> Under the conditions of these 2-year inhalation studies, methyl bromide caused degenerative changes in the cerebellum and cerebrum, myocardial degeneration and cardiomyopathy, sternal dysplasia, and olfactory epithelial necrosis and metaplasia. There was no evidence of carcinogenic activity for methyl bromide in male and female B6C3F1 mice. Toxic effects persisted although exposure to methyl bromide in the 100 ppm group terminated after 20 weeks.

Dr. Ashby, a principal reviewer, agreed with the conclusions. He said that in view of the fact that methyl bromide is a methylating agent and clearly genotoxic *in vitro* and *in vivo* it was quite surprising that the chemical had no neoplastic effects.

Dr. Zeise, the second principal reviewer, agreed with the conclusions. She was pleased with the dose level selection noting that even though the Maximum Tolerated Dose (MTD) apparently was exceeded in male mice, the other two dose groups were adequate for evaluation.

Dr. Longnecker, the third principal reviewer, agreed with the conclusions. However, he wondered why more import was not given to the category of the overall increased incidence of animals with malignant tumors which was significant in low dose male mice (P=0.01) compared with controls. Dr. Eustis replied that the increase in malignant tumors was primarily due to an increase in alveolar bronchiolar carcinomas which was balanced off by a decrease in adenomas. Since the adenomas and carcinomas are a morphologic continuum, and further, since the increased incidence of malignant neoplasms was not observed in the mid or high dose groups, the staff considered this not to be a real effect.

Dr. Yang reported that a chronic study by Dutch workers in Wistar rats had given negative results. He said more details on the rat study would be included in the report. Dr. Silbergeld said the central nervous system (CNS) pathology was rather surprising in that it was noted only at the high dose while other data suggest that there is much more of a dose-related trend in the overt neurotoxicity of the chemical. She said there should be caution introduced in the text in interpreting the neurotoxicity results, both behaviorally and pathologically in that there are pitfalls in some of the tests used to measure neurotoxicity. There was sentiment among some of the members for adding incidence rates for significant non-neoplastic lesions to the summary table in the Abstract. Dr. Scala commented that the sense of the Panel was that they wanted the Abstract and the text to reflect a report on an important neurotoxicant and the staff had indicated this perspective would be given.

Dr. Ashby moved that the Technical Report on methyl bromide be accepted with the revisions discussed and the conclusions as written for male and female mice, no

evidence of carcinogenic activity. Dr. Zeise seconded the motion, which was accepted unanimously with twelve votes.

Monochloroacetic Acid. Dr. K.M. Abdo, NIEHS, Staff Scientist, introduced the toxicology and carcinogenesis studies of monochloroacetic acid by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on non-neoplastic lesions that were observed. The conclusions were that:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of monochloroacetic acid for male or female F344/N rats given 15 or 30 mg/kg in water by gavage for 104 weeks. There was no evidence of carcinogenic activity of monochloroacetic acid for male or female B6C3F1 mice given 50 or 100 mg/kg in water by gavage for 104 weeks.
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> Monochloroacetic acid administration was associated with inflammatory lesions of the nasal mucosa, metaplasia of the olfactory epithelium, and squamous cell hyperplasia of the forestomach in male and female mice.

Dr. Davis, a principal reviewer, agreed with the conclusions. He commented that for female mice an MTD may not have been reached in that there was only a 6% difference between terminal control and high dose group animal body weights. Furthermore, for weeks 53 to 103, the difference in mean body weight for these two groups was 9%. Dr. Eustis explained that even if the decrement in body weight is less than 10%, if there is a consistent decrement over a long period of time as in this case then we feel fairly certain that it is a toxic effect. Dr. J. Haseman, NIEHS, added that nasal cavity and forestomach non-neoplastic effects in this study suggested an MTD had been achieved.

Dr. Longnecker, the second principal reviewer, agreed with the conclusions.

Dr. Ashby, the third principal reviewer, agreed with the conclusions. He noted that the genetic toxicity profile continues a trend established in earlier studies, namely, no structural alert, no mutagenicity to Salmonella or clastogenicity to Chinese hamster ovary cells, but increases in the L5178Y and sister chromatid exchange (SCE) assays. This confirmed again that the latter two protocols were not correlated at all with in vivo carcinogenicity.

There was some discussion about the forestomach lesions in mice, especially the increased incidence of squamous cell papillomas in females and whether significantly increased incidence of hyperplasia in the high dose group reflected a preneoplastic effect or rather focal irritation due to gavage with an irritating substance. The staff position was in support of the latter.

Dr. Davis moved that the Technical Report on monochloroacetic acid be accepted with the revisions discussed and the conclusions as written for male and female rats and mice, no evidence of carcinogenic activity. Dr. Longnecker seconded the motion, which was accepted unanimously with eleven votes.

Probenecid. Dr. K.M.Abdo, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of probenecid by discussing the uses, describing the experimental design, reporting on survival, body weight and liver weight effects in rats and mice, and commenting on the only compound-related lesions, those being hepatocellular tumors in female mice. The conclusions were that:

> Under the conditions of these 2-year studies, there was no evidence of carcinogenic activity of probenecid for male or female F344/N rats receiving 100 or 400 mg/kg in corn oil by gavage for 103 weeks. There was no evidence of carcinogenic activity for male B6C3F1 mice given 100 or 400 mg/kg probenecid in corn oil by gavage for 103 weeks. There was some evidence of carcinogenic activity of probenecid for female B6C3F1 mice based on an increased incidence of hepatocellular neoplasms.

Dr. Carlson, a principal reviewer, agreed with the conclusions. He commented on the statement that no chemical-related toxic effects were observed in male or female rats as being contradictory to statements in the results that "the moribund condition of these animals was presumed to be the result of chemical toxicity" and "these deaths were therefore presumed to be related to chemical toxicity." Dr. Scala said the issue of moribund animals and the relationship of their condition to chemical toxicity needed to be clarified in the report.

Dr. Garman, the second principal reviewer, agreed with the conclusions. However, he questioned why for female mice, hepatocellular adenomas and carcinomas were combined in the summary table, when the frequency of carcinomas was obviously not treatment related. Dr. Eustis, NIEHS, said the carcinoma would be separated out in the table.

Dr. Gold, the third principal reviewer, agreed with the conclusions. She said that if the level of some rather than clear evidence in female mice was because there was an increase only in benign tumors, then the conclusion should indicate this. Dr. Abdo said the conclusion would say hepatocellular adenomas. Dr. Gold commented that assigning cause of death to moribund sacrificed animals is extremely difficult and may not be accurate.

Dr. Carlson moved that the Technical Report on probenecid be accepted with the conclusions as written for male and female rats and male mice, no evidence of carcinogenic activity, and for female mice, some evidence of carcinogenic activity, with the last sentence being changed to emphasize that the conclusion was based on benign neoplasms. Dr. Garman seconded the motion, which was accepted unanimously with eleven votes.

Titanocene Dichloride. Dr. J.K. Dunnick, NIEHS, NTP Staff Scientist, introduced the toxicology and carcinogenesis studies of titanocene dichloride by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related non-neoplastic and neoplastic lesions in male and female rats. The conclusions were that:

> Under the conditions of these 2-year studies, there was equivocal evidence of carcinogenic activity of titanocene dichloride for male and female F344/N rats based on a marginal increase in the incidence of squamous cell neoplasms of the forestomach. Nonneoplastic lesions associated with the administration of titanocene dichloride for up to 2 years included erosions and inflammation of the gastric mucosa, hyperplasia and metaplasia of the fundic glands with fibrosis of the lamina propria in the glandular stomach, and acanthosis (hyperplasia) and hyperkeratosis of the forestomach epithelium.

Dr. Gold, a principal reviewer, agreed with the conclusion in female rats but thought consideration should be given to changing the conclusion in male rats to some evidence of carcinogenic activity. The incidences of squamous cell papillomas of the forestomach were: controls, 0/60; low dose, 4/60; high dose, 1/60. Additionally, there was a carcinoma in the low dose. She based her proposed change on the rarity of these tumors, 0.3% in both male and female historical controls, with only one carcinoma in the NTP historical data base, the significantly reduced survival in high dose male rats, and the significant increases in forestomach hyperplasias in both low and high dose groups. Dr. Gold asked that the NTP consider routinely reporting incidences for nonneoplastic lesions that are selected to be listed in the summary table in the Abstract. Dr. Gold questioned the rationale for testing this chemical given the lack of human exposure.

Dr. Silbergeld, the second principal reviewer, agreed with the conclusions. In view of a reference reporting increased incidence of lung tumors in rats following inhalation exposure to titanocene, she wondered if there was a potential for inhalation exposure in the workplace. Dr. J. Haartz, NIOSH, said that to her knowledge current use is only in research laboratories.

Dr. Hayden, the third principal reviewer, agreed with the conclusion for female rats but also thought consideration should be given to changing the conclusion for male rats to some evidence of carcinogenic activity. He based this on: (1) a statistically significant increase of forestomach squamous papillomas that exceeded those found in study controls, historical controls at the study site, and NTP overall historical controls, (2) a lack of inference that stomach tumor incidence in study controls was below that expected for historical controls, and (3) a possibility that an increased incidence of forestomach neoplasia would have been seen in high dosed males had more survived to term. Dr. S. Eustis, NIEHS, responded to the reviewers proposal to change the level of evidence in male rats. He noted that there was not a dose reponse in male rats, the doses given were sufficient to cause considerable toxicity in the forestomach, the numbers of tumors were few, and perhaps most importantly, all but one of the papillomas (in both sexes) occurred at the limiting ridge which is where all of the forestomach toxicity occurred. Further, the hyperplasias observed represented the kind that one sees as a regenerative response to toxicity rather

than a preneoplastic lesion. Dr. Zeise proposed that the squamous cell papillomas of the palate seen in one high dose male rat and one high dose female rat be included in discussion of the other squamous cell tumors of the gastrointestional tract.

Dr. Hayden moved that the Technical Report on titanocene dichloride be accepted with the revisions discussed and the conclusions as written for male and female rats, equivocal evidence of carcinogenic activity. Dr. Goodman seconded the motion, which was accepted unanimously with eleven votes.

TOXICITY STUDIES

Antimony Potassium Tartrate. Dr. M.P. Dieter, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of antimony potassium tartrate by reviewing the rationale, experimental design, and results. Antimony potassium tartrate (APT) was administered to groups of F344/N rats and B6C3F1 mice of both sexes for 14 days in drinking water or by i.p injection for 16 days to determine the most appropriate route of exposure for further studies. As APT was poorly absorbed and relatively nontoxic by the oral route and human exposure as an antischistosomal agent was by injection, the i.p. route was chosen for 90-day studies. Besides pathology and histopathology, a large number of supplemental clinical pathology tests and tissue antimony assays were included. Rats were more sensitive than mice to the toxic effects of APT, exhibiting dose-related mortality and reduction in body weight. There were no clinical signs of toxicity nor gross or microscopic lesions in mice that could be attributed to toxicity of APT. Dose-related increased concentrations of antimony were detected in the blood, liver, kidney, spleen, and heart of rats, and in the liver and spleen of mice. In rats, hepatocellular degeneration and necrosis was associated with dose-related elevations in activities of the liver-specific serum enzymes, sorbitol dehydrogenase and alanine aminotransferase. A treatment-related degeneration of the kidney was present in three high dose male rats; findings that were not reflected in increases in urinary enzyme markers for tubular cell injury.

Dr. Klaassen, a principal reviewer, thought this was a good study and report on the short-term toxicity of antimony potassium tartrate. He questioned why the chemical was studied since only a very small number of people in the U.S. are exposed.

Dr. Davis, a second principal reviewer, agreed that this was a well designed and clearly reported study. He asked why deionized water was the vehicle for 14-day studies while physiological saline was used for 13-week studies. Dr. Dieter said deionized water was used in drinking water studies to avoid confounding effects from other cations or anions while the 13-week studies were by injection so physiological saline was used to maintain osmotic equilibrium.

Dr. Carlson noted that the Salmonella assay was negative as expected and suggested that a mammalian cell genetic toxicity assay should have been done as the data from the literature indicated a likely positive outcome.

Castor Oil. Dr. R.D. Irwin, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of castor oil by reviewing the experimental design and results. Castor oil was administered by the dosed feed route in concentrations as high as 10% to groups of F344/N rats and B6C3F1 mice of both sexes for 13-weeks. Exposure to concentrations as high as 10% did not affect survival or body weight gains of rats or mice. Liver weights were increased in male rats at the high dose and in male and female mice receiving diets containing five or 10%, although treatment-related histopathologic lesions were not present in any organs in rats or mice. No significant changes were noted in male or female reproductive end points of rats or mice. Thus, no significant adverse effects of castor oil administration were noted in the NTP studies.

Dr. Davis, the principal reviewer, said that this was a well designed study and a clear concise report of high technical quality which met the requirements and expectations for an NTP document.

Cresols. Dr. D.D. Dietz, formerly NIEHS, NTP Study Scientist, introduced the short-term toxicity studies of cresols by reviewing the rationale, experimental design, and results. In 28-day toxicity studies, F344/N rats and B6C3F1 mice of both sexes were given o-cresol, m-cresol, p-cresol, or m/p-cresol (60:40) at concentrations from 300 to 30,000 ppm in the diet. Mice, who ingested larger quantities of cresols per unit body weight, developed a greater toxic response than rats. Treatment-related microscopic changes occurred at more tissue sites and at lower levels of exposure among p- and m/p-cresol treated rats and mice compared to groups receiving o- and m-cresol. In 13-week studies, o-cresol or m/p-cresol were added to the diet in concentrations as high as 30,000 ppm to F344/N rats and 20,000 ppm (o-cresol) or 10,000 ppm (m/p-cresol) to B6C3F1 mice. No deaths of rats or mice could be associated with chemical exposure. Results of microscopic analyses were consistent with findings in 28-day studies, and showed mild bone marrow hypocellularity in rats and forestomach hyperplasia in mice given higher concentrations of o-cresol. There was little evidence of increases in toxic effects for o- or m/p-cresol from 28 days to 13 weeks, and the isomers exhibited a generally similar pattern of toxicities in rats and mice. Histopathologic changes, including bone marrow hypocellularity, irritation to the gastrointestinal tract and nasal epithelia, and atrophy of female reproductive organs, occasionally occurred at 10,000 ppm, but were more common at the high dose of 30,000 ppm.

Dr. Carlson, a principal reviewer, said the text dealt well with a massive amount of data and the general conclusions drawn and target tissues identified appeared to be justified by data. He asked if dermal exposure was considered since the major concern, use and contacts are to the skin. Dr. Dietz responded that this was part of a Superfund initiative concerned with groundwater so oral exposure was indicated. Dr. Carlson complained that it was sometimes difficult to judge whether incidence or severity was being discussed in the text, and asked that these distinctions be clarified.

Dr. Garman, a second principal reviewer, said the report presents a large amount of information about as well as the format will allow. The literature reviews appeared to be thorough and the discussion section did a good job of discussing the treatment-associated lesions.

Both Dr. Carlson and Dr. Scala commented on the importance and utility of these short-term in vivo studies. Dr. Scala said it would be most useful if a sentence or two could be added giving a comparison of the relative toxicity of the three isomers.

Ethylbenzene. Dr. P.C. Chan, NIEHS, NTP Staff Scientist, introduced the short-term toxicity studies of ethylbenzene by reviewing the rationale, experimental design, and results. Ethylbenzene vapor was administered by the inhalation route in concentrations ranging from 100 to 1000 ppm to groups of F344/N rats and B6C3F1 mice of both sexes for 13-weeks. No rats or mice died during the 13-week exposure. Absolute and relative kidney, liver, and lung weights were increased in exposed rats, and liver weights in exposed mice but with no accompanying chemically related histopathologic changes. No changes were observed in the evaluation of sperm or vaginal cytology in rats or mice, and ethylbenzene was not mutagenic in bacterial or mammalian cell assays. Thus, there appeared to be only minimal evidence of toxicity in rats and mice exposed to ethylbenzene by inhalation at up to 1000 ppm for 13 weeks.

Dr. Hayden, a principal reviewer, commented on the reported decreased serum alkaline phosphatase activity being related to reduced food and water intake, and wondered if it was the intestinal isozyme or the liver isozyme. Dr. M. Thompson, NIEHS, said it was the intestinal isozyme and he didn't believe the decreases in activity were related to chemical effects.

Dr. Klaassen, a second principal reviewer, thought this a well-conducted study which demonstrated only minimal toxicity for the chemical.

Dr. Carlson asked that serious consideration be given to defining and assigning severity scores for appropriate lesions such as those reported as inflammation in the lungs of rats exposed to ethylbenzene. Dr. M. Elwell, NIEHS, agreed.

Body Weight, Survival and Tumor Trends in F344 Rats and Dietary Considerations to Modify These Trends - Dr. G.N. Rao, NIEHS, said he wanted to present an update on time trends for growth, body weight, survival and tumors in Fischer 344 (F344) rats and plans to modify or even reverse these trends by diet modifications (Attachment 1). One hundred and fifty diet control groups of male rats and 152 groups of female rats were evaluated over a 12 year period (1971 to 1982) (Attachment 1, Slide 3).

He reported that male and female rats in more recent studies grew faster and attained a higher body weight than rats from earlier studies during this period (Attachment 1, Slides 4-7), while there was a corresponding decreasing trend for survival, more marked in male rats but significant for both males and females (Attachment 1, Slides 8-9).

Dr. Rao then presented time trends for various tumors. There were increased incidences of pituitary tumors and hematopoietic system neoplasms (leukemias) in rats of both sexes, and of mammary tumors in females. There was a highly statistically significant association with body weight for pituitary and mammary tumors but no association for leukemias (Attachment 1, Slides 10-17). He commented that other laboratories are seeing parallel increases in body weights and tumor incidences and decreases in survival in F344 as well as Sprague-Dawley rats.

Dr. Rao cited reasons for increased body weight and faster growth in F344 rats that included elimination of infectious diseases, improved diet, decreased stress and energy loss, genetic changes, and selection of faster-growing progeny (Attachment 1, Slide 23). He cited increases in leukemia, pituitary and mammary tumors, and kidney disease as contributing factors for decreasing survival (Attachment 1, Slide 24).

He described NTP dietary modifications from the standard diet (NIH-07) previously used that were made in 1988 (Attachment 1, Slide 25), and aimed at improving animal survival through reductions in protein and fat and increases in fiber. However, on this diet, survival decreased. There appeared to be a correlation with the type of fat used (usually soy oil). Thus, in 1990 a new diet was formulated containing primarily corn oil as the lipid component (Attachment 1, Slide 32). Along with further increases in fiber to reduce caloric density and lower protein composition, the aim is to effect increased survival in rats by decreasing severity of nephropathy, decreasing incidence of leukemia, and decreasing incidences of pituitary and mammary tumors (Attachment 1, Slide 31).

Studies on Caloric Restriction in F344 Rats and B6C3F1 Mice at the National Center for Toxicological Research (NCTR) - Dr. William Allaben, NCTR, said the project on caloric restriction (PCR) was designed in collaboration with the National Institute on Aging (NIA) as a nine-year study looking at several strains of rats and mice. In this study, the NIA is primarily concerned with biomarkers of aging while the NCTR is concerned with exploring the effects and mechanisms of caloric restriction on toxicity. Animals are maintained on 40% restriction or 60% of ad lib fed controls.

Dr. Allaben's remarks were confined to the findings in F344 rats and B6C3F1 mice, the strains commonly used in NTP studies. As expected, there were reductions in body weight gain and significantly enhanced survival in both rats and mice. There was also a reduction in the degree of severity of nephropathy at 24 months in both sexes of rats. While incidences of pituitary gland tumors were reduced in calorically restricted animals of both sexes, leukemia rates did not seem to be inhibited by a reduction in calories or a concomitant reduction in body weight.

Dr. Allaben concluded by discussing studies designed to examine effects of caloric restriction on the expression and mechanisms of neoplastic response of the liver to aflatoxin B1.

ATTACHMENT 1

Slide 1

BODY WEIGHT, SURVIVAL AND TUMOR TRENDS IN F344 RATS AND DIETARY CONSIDERATIONS TO MODIFY THESE TRENDS

G. N. RAO

Slide 2

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Growth, Body Weight, Survival, and Tumor Trends in F344/N Rats During an Eleven-Year Period*

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[3]*Pathology Associates, Inc., Durham, North Carolina*

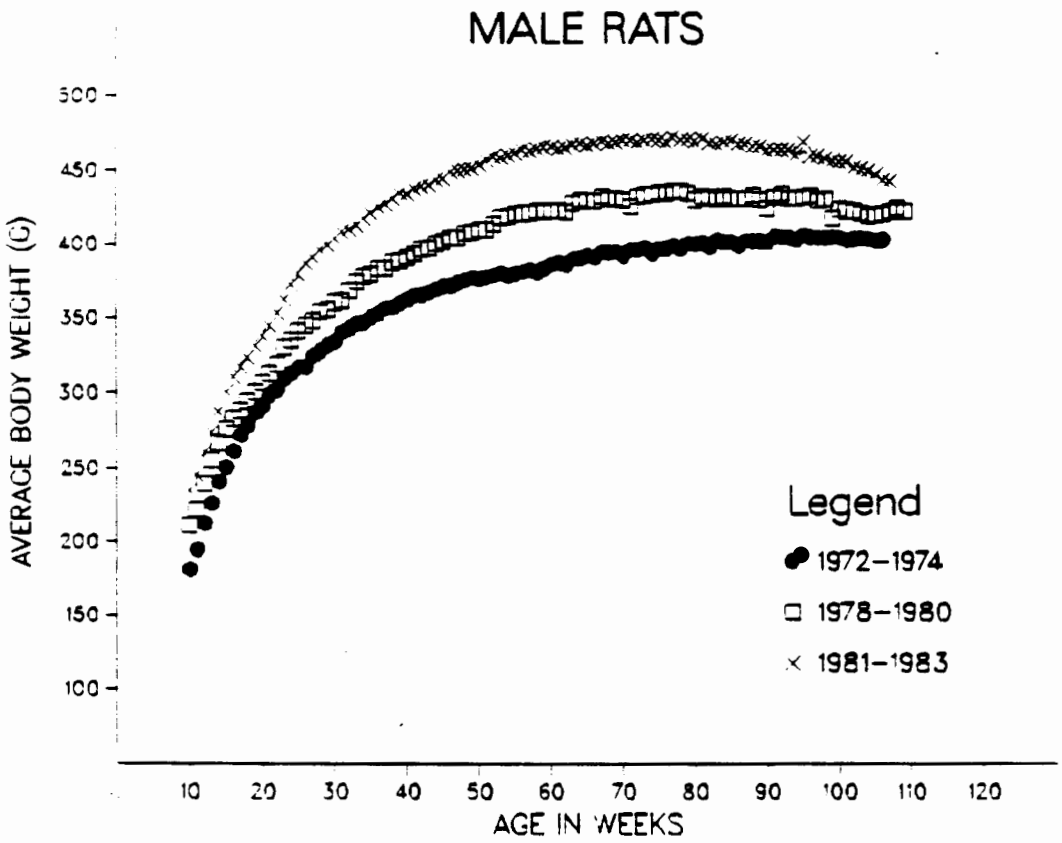
Slide 3

TABLE I. Number of studies by year and testing laboratory included in this investigation.

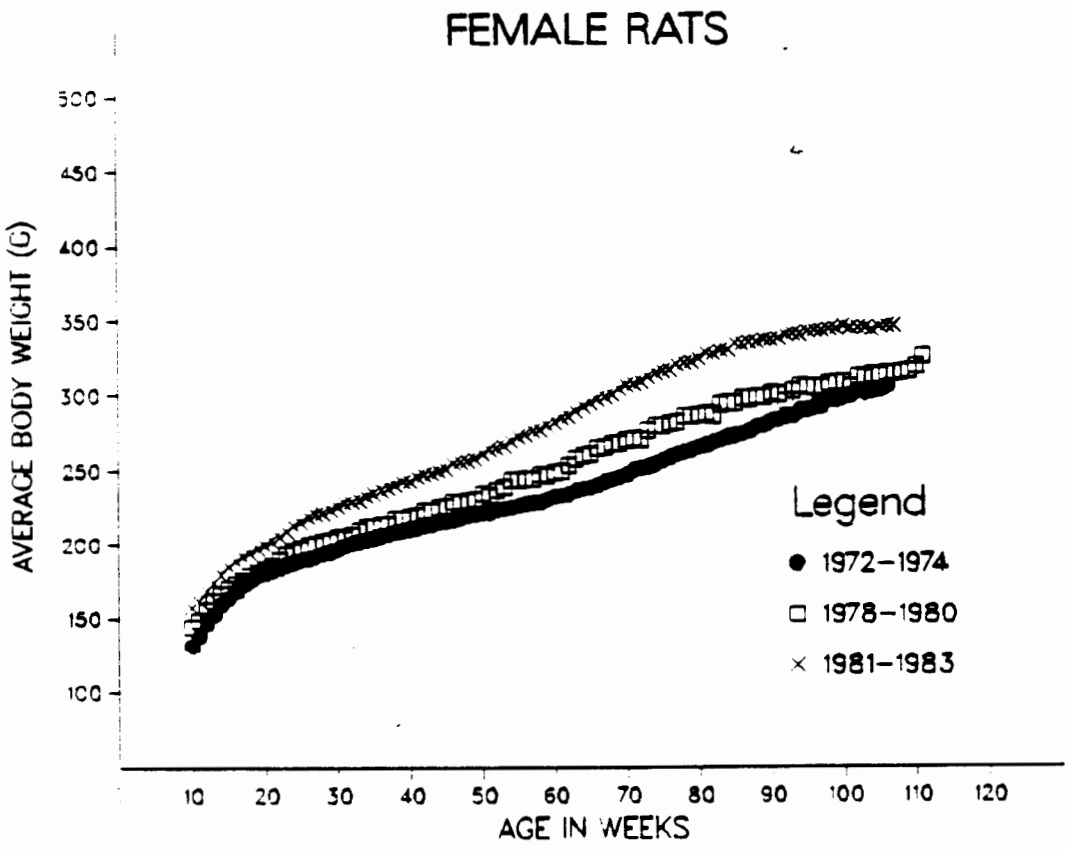
Year[a]	No. of Studies Male	No. of Studies Female	Laboratory	No. of Studies Male	No. of Studies Female
1971	3	3	1	9	9
1972	22	22	2	2	2
1973	42	42	3	23	24
1974	10	10	4	9	9
1975	4	4	5	6	6
1976	9	9	6	32	32
1977	13	15	7	29	30
1978	12	12	8	1	1
1979	11	11	9	1	1
1980	10	10	10	8	8
1981	11	11	11	27	27
1982	3	3	12	3	3
Total	150	152		150	152

[a]Year of study start. Studies ended two years later (e.g. 1971 starts ended in 1973, 1980 starts in 1982).

Slide 4 (Figure 1)



Slide 5 (Figure 2)



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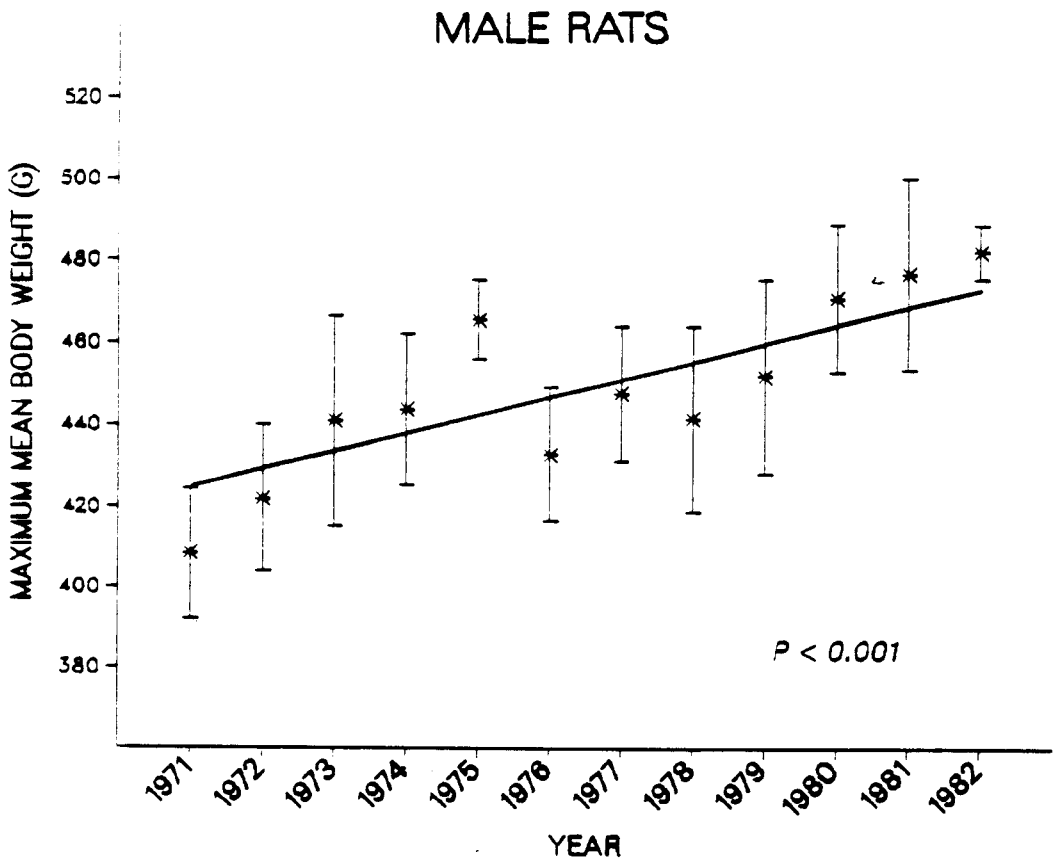

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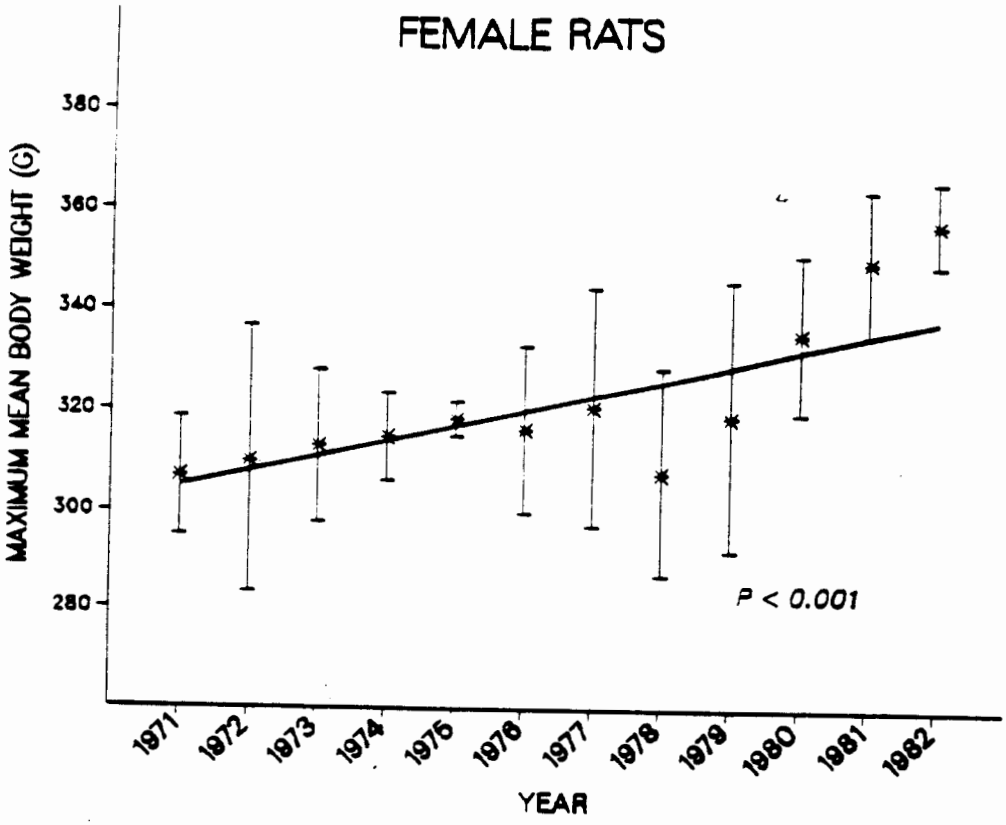
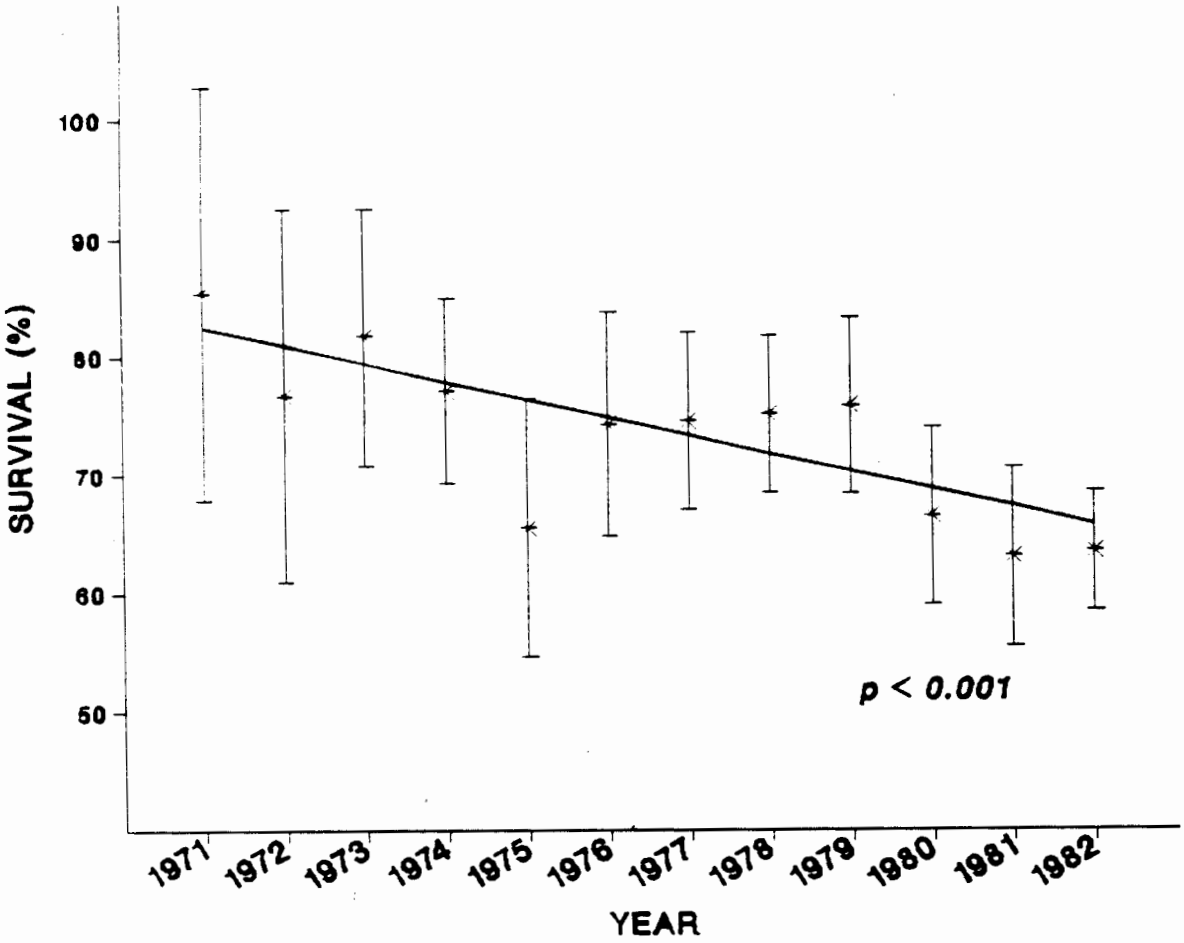


Slide 8 (Figure 5)

Male Rats



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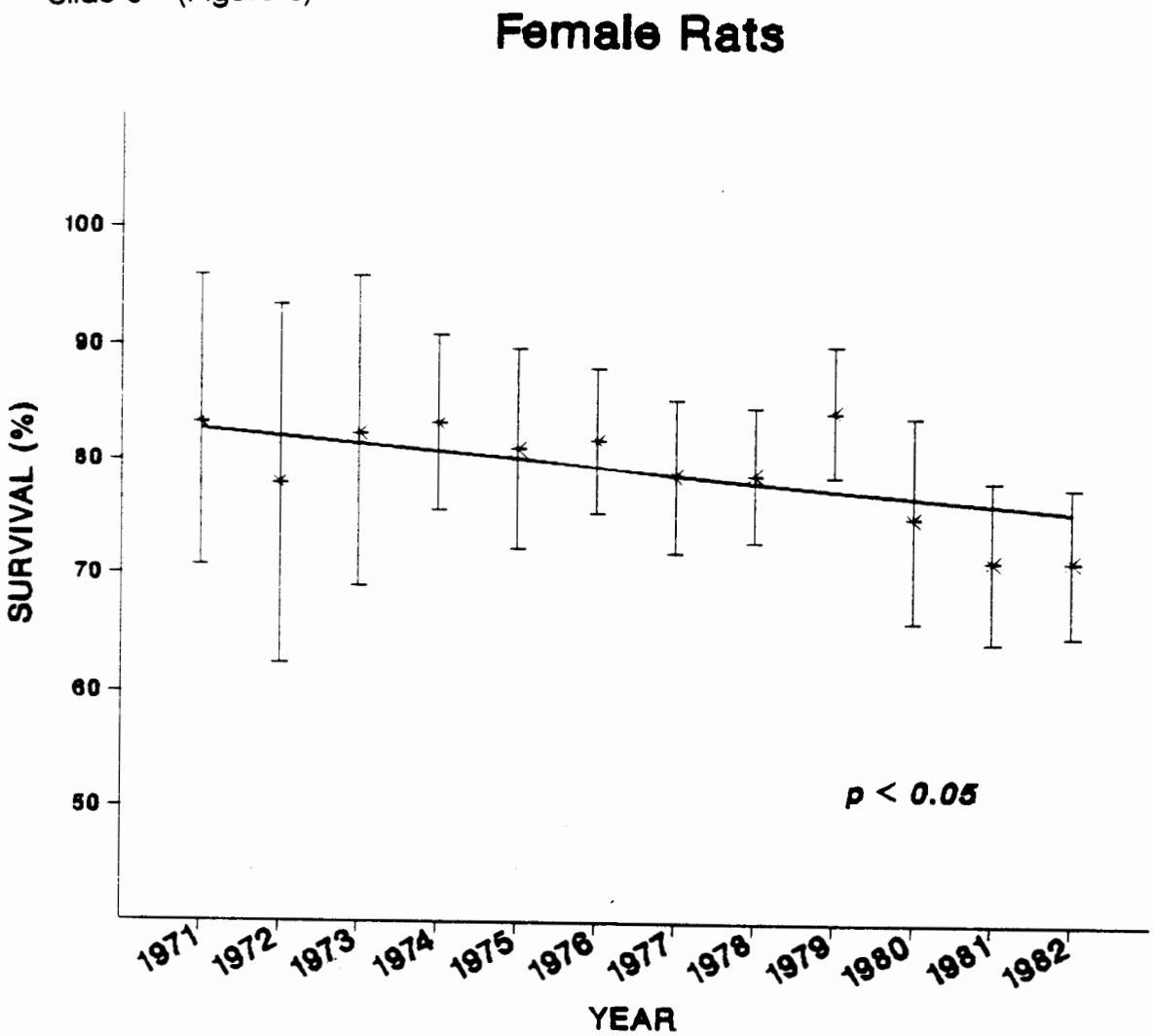

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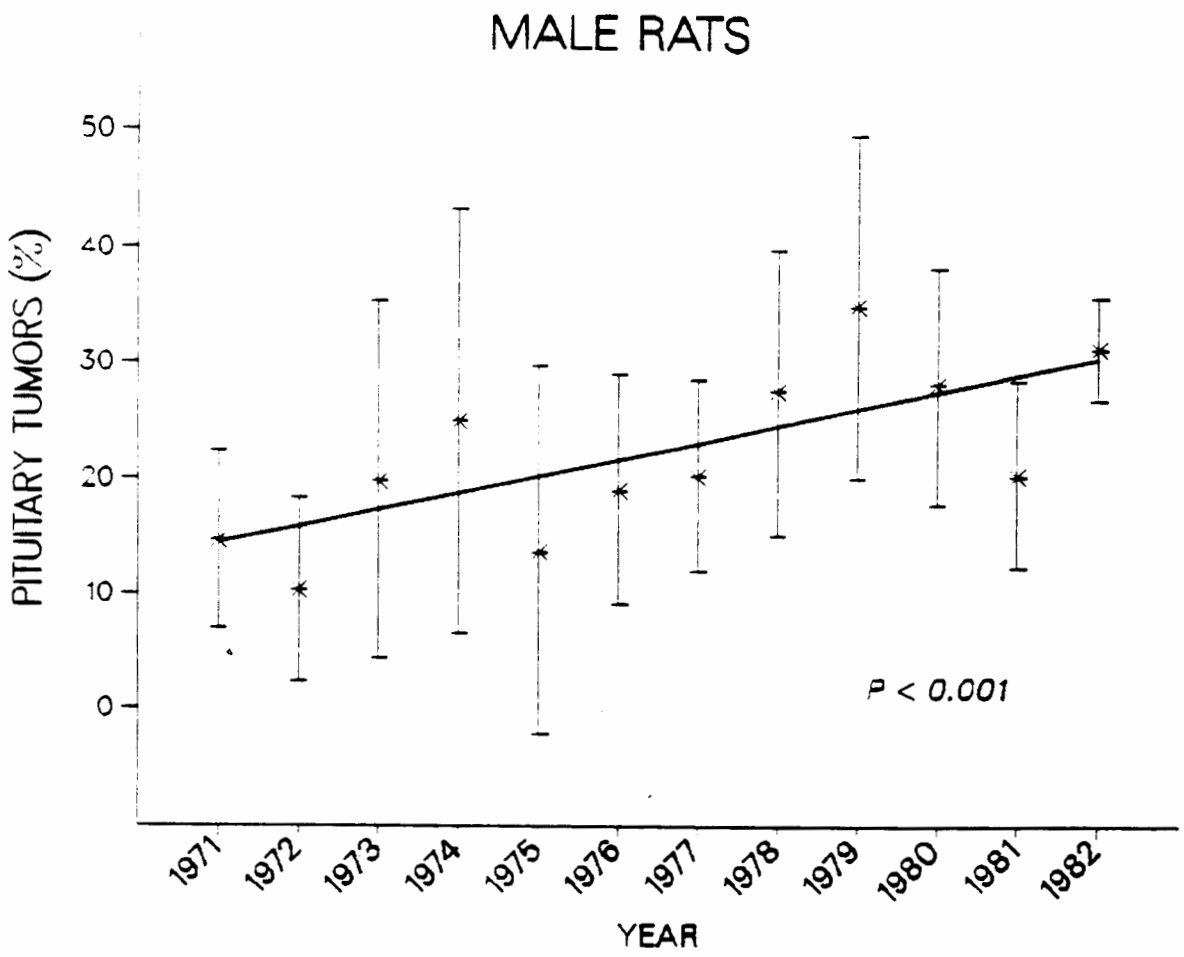

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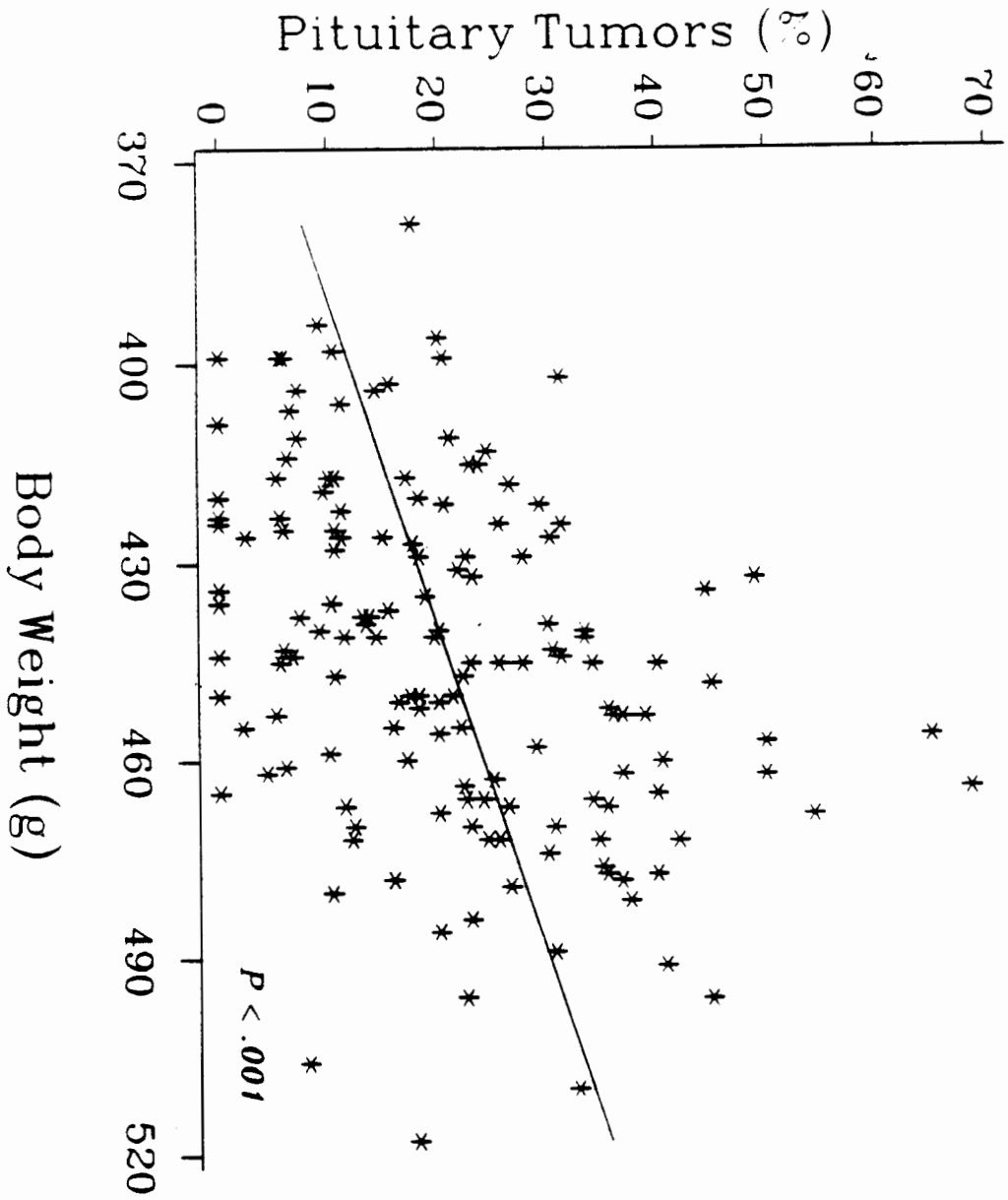

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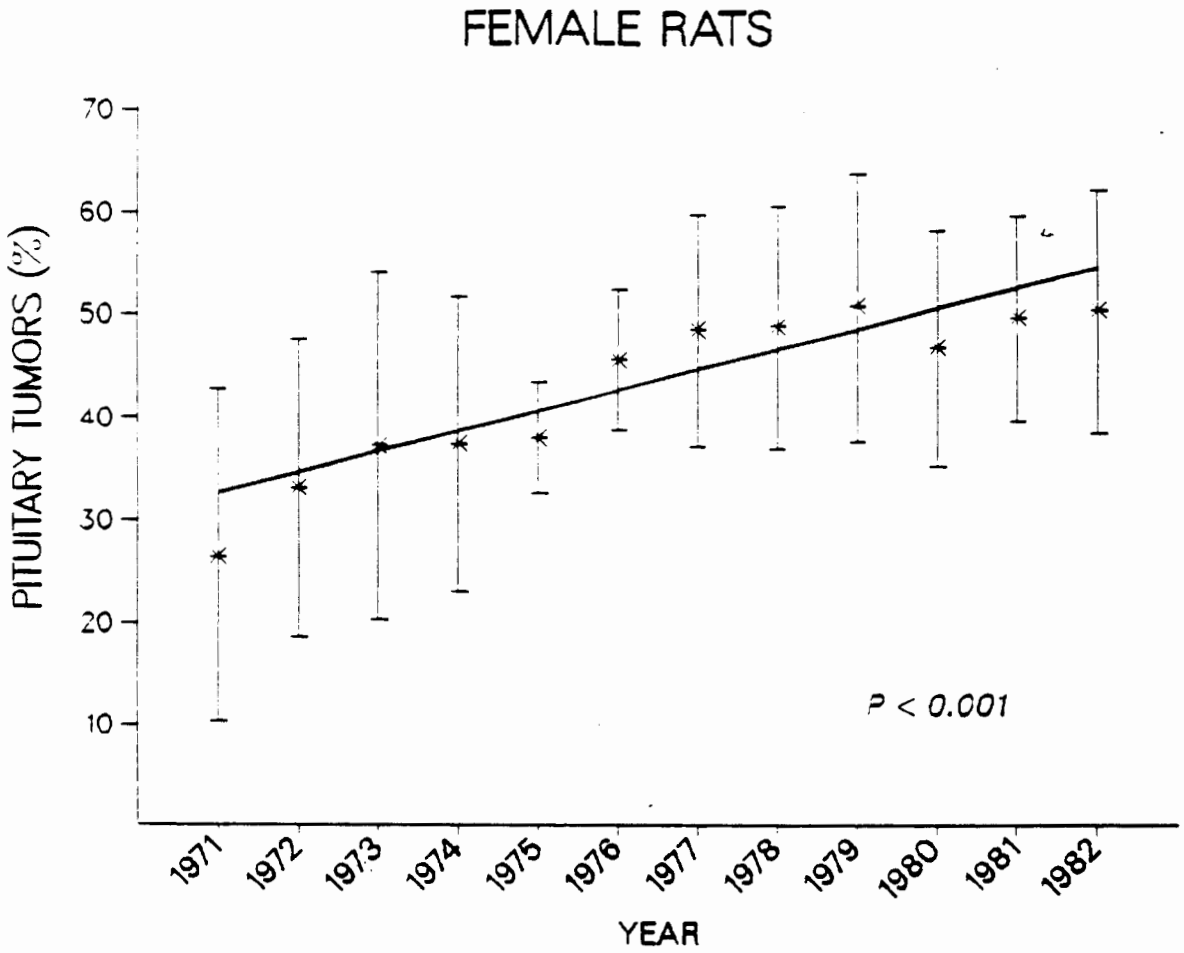

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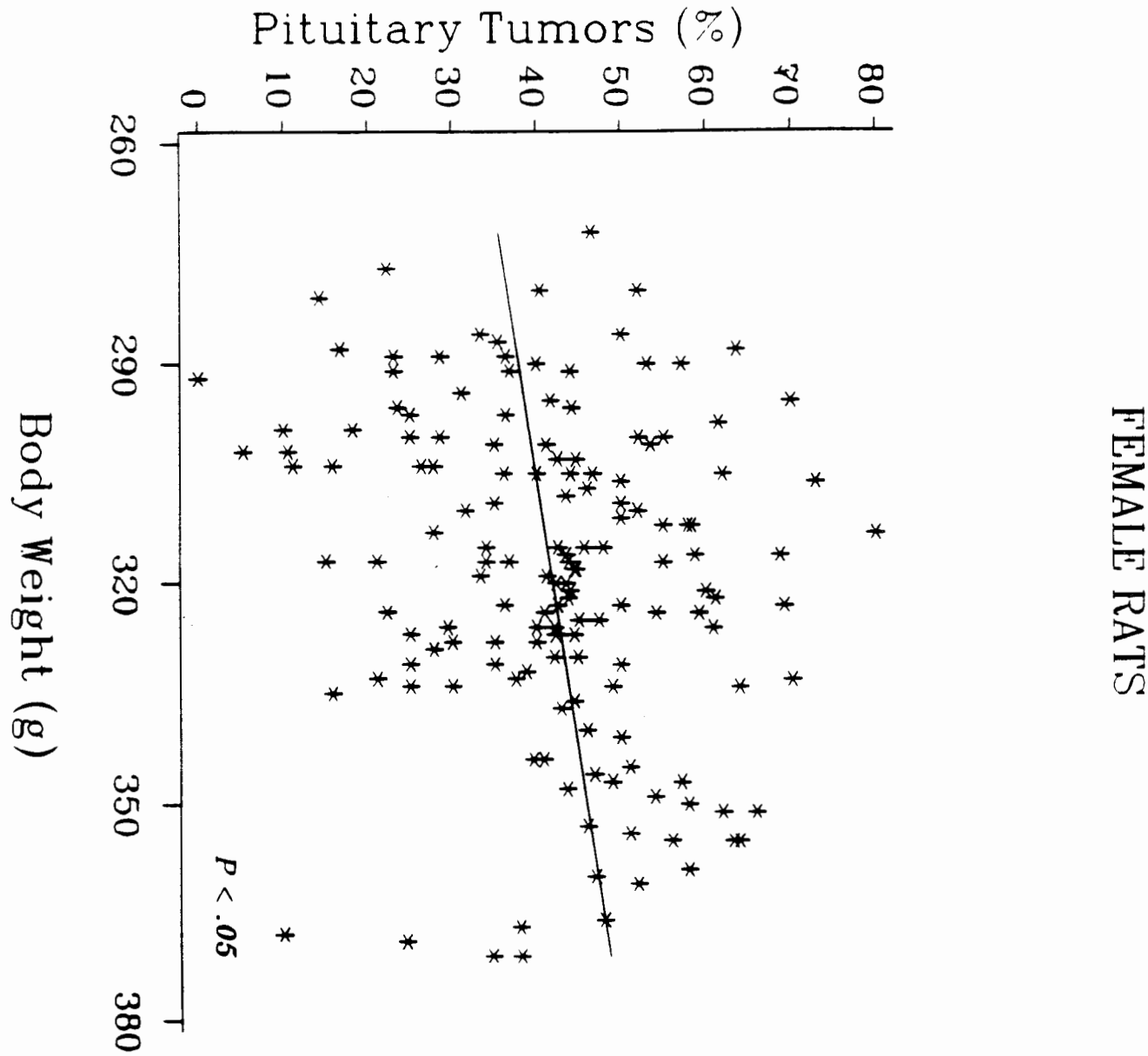

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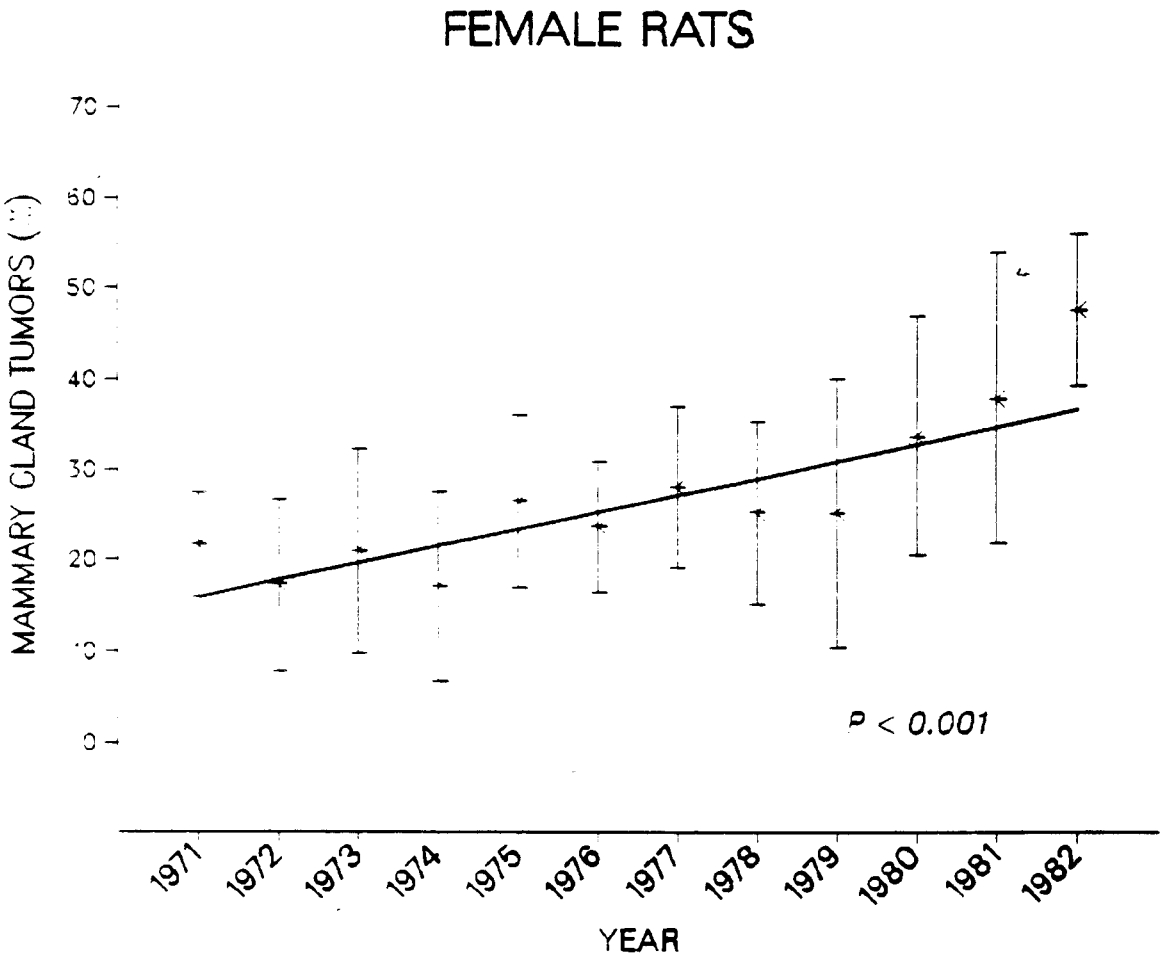

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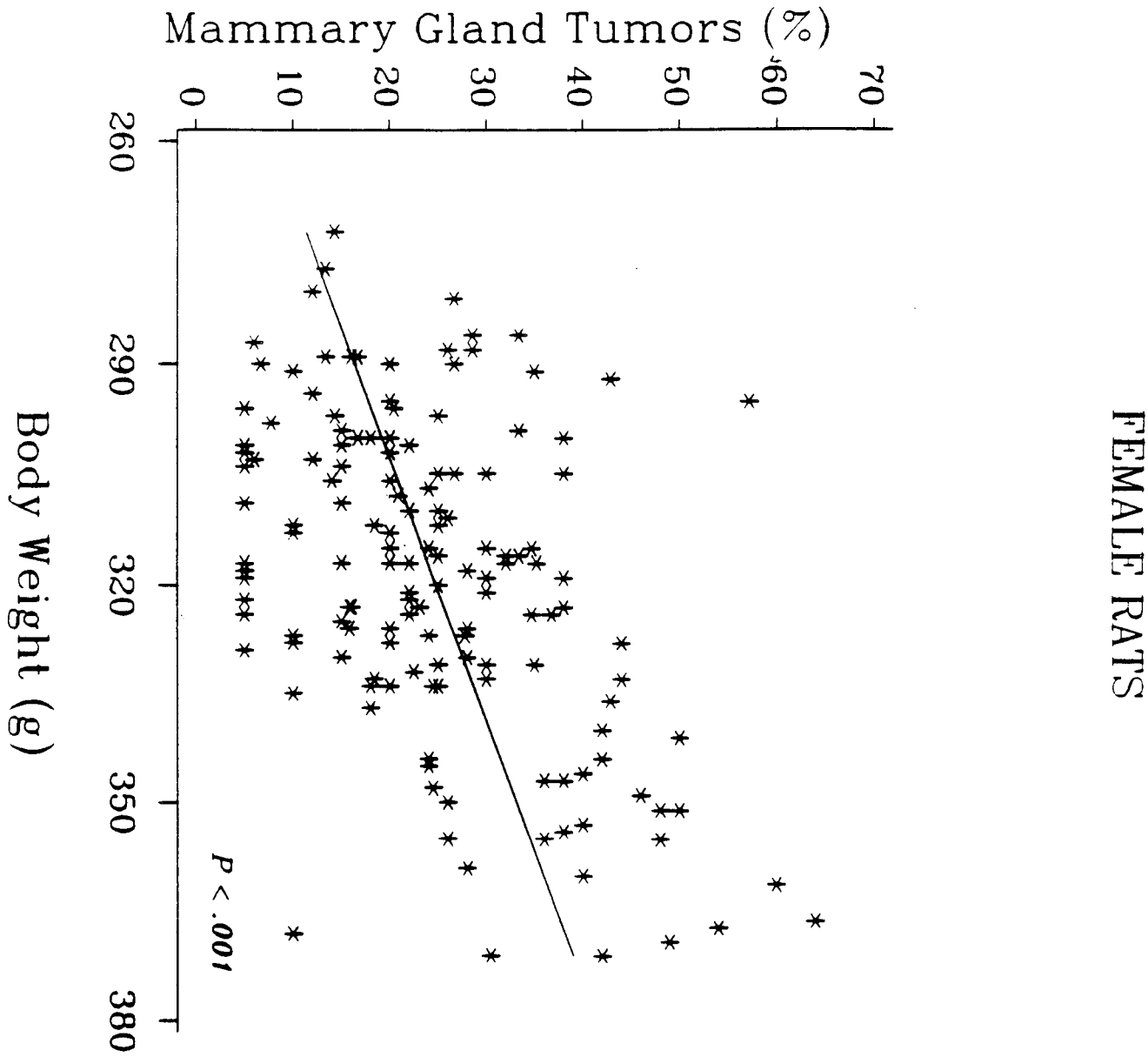

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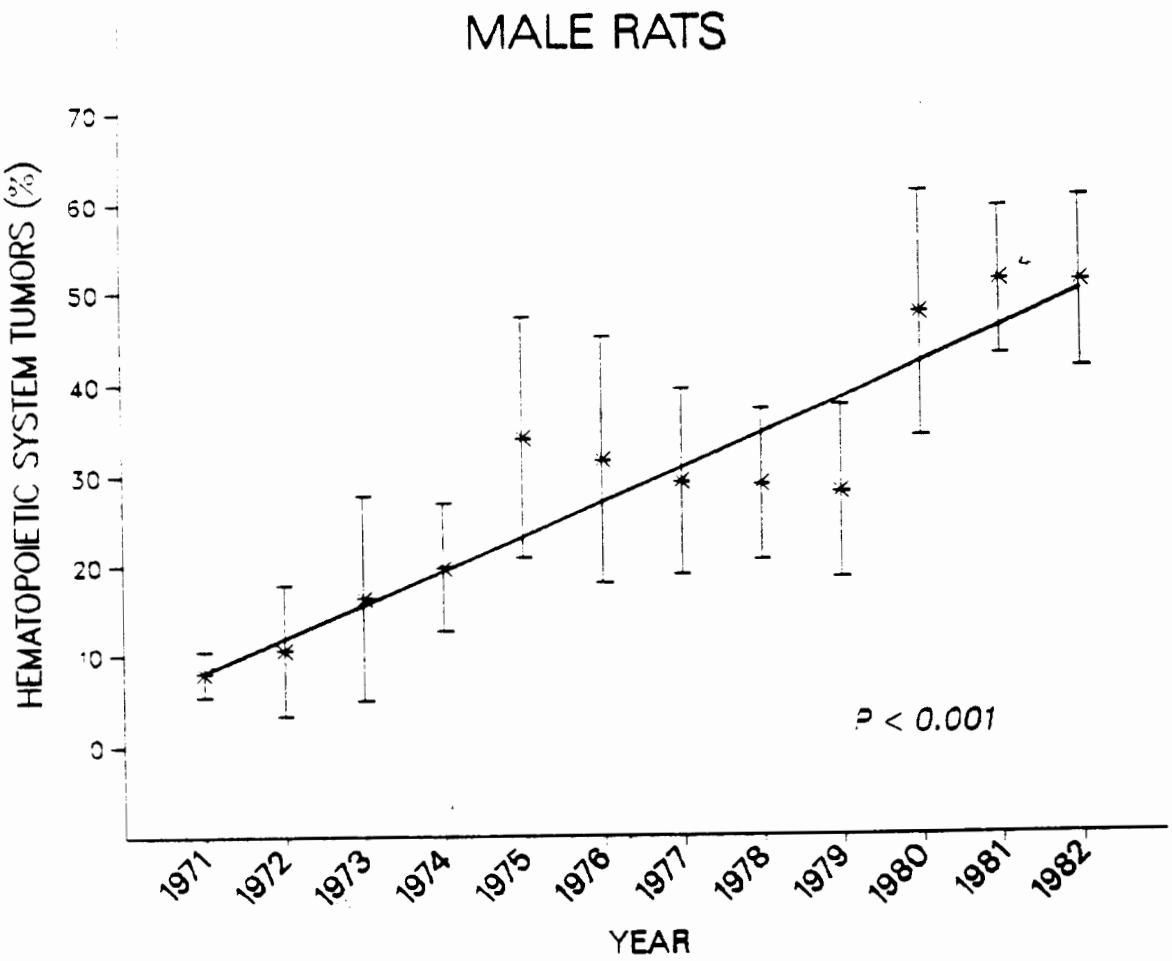

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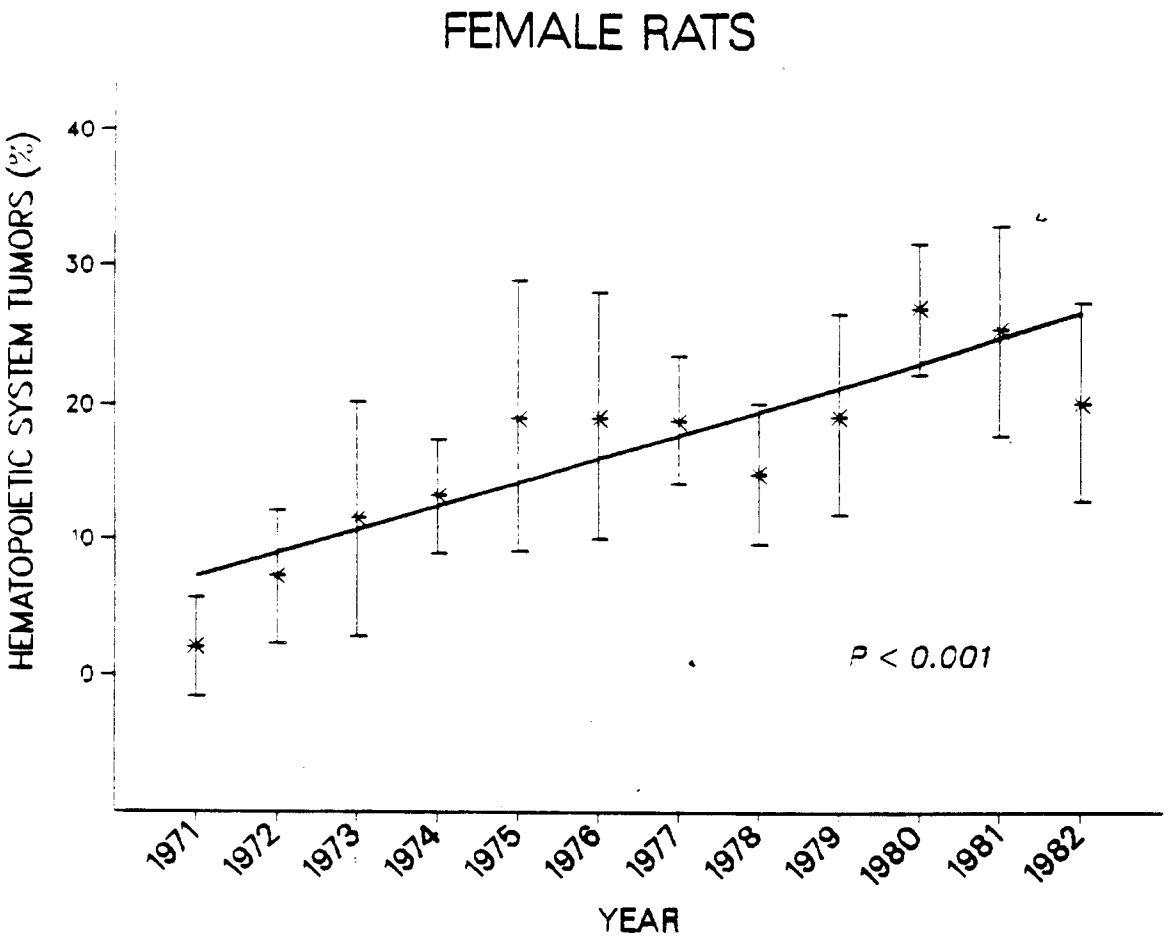

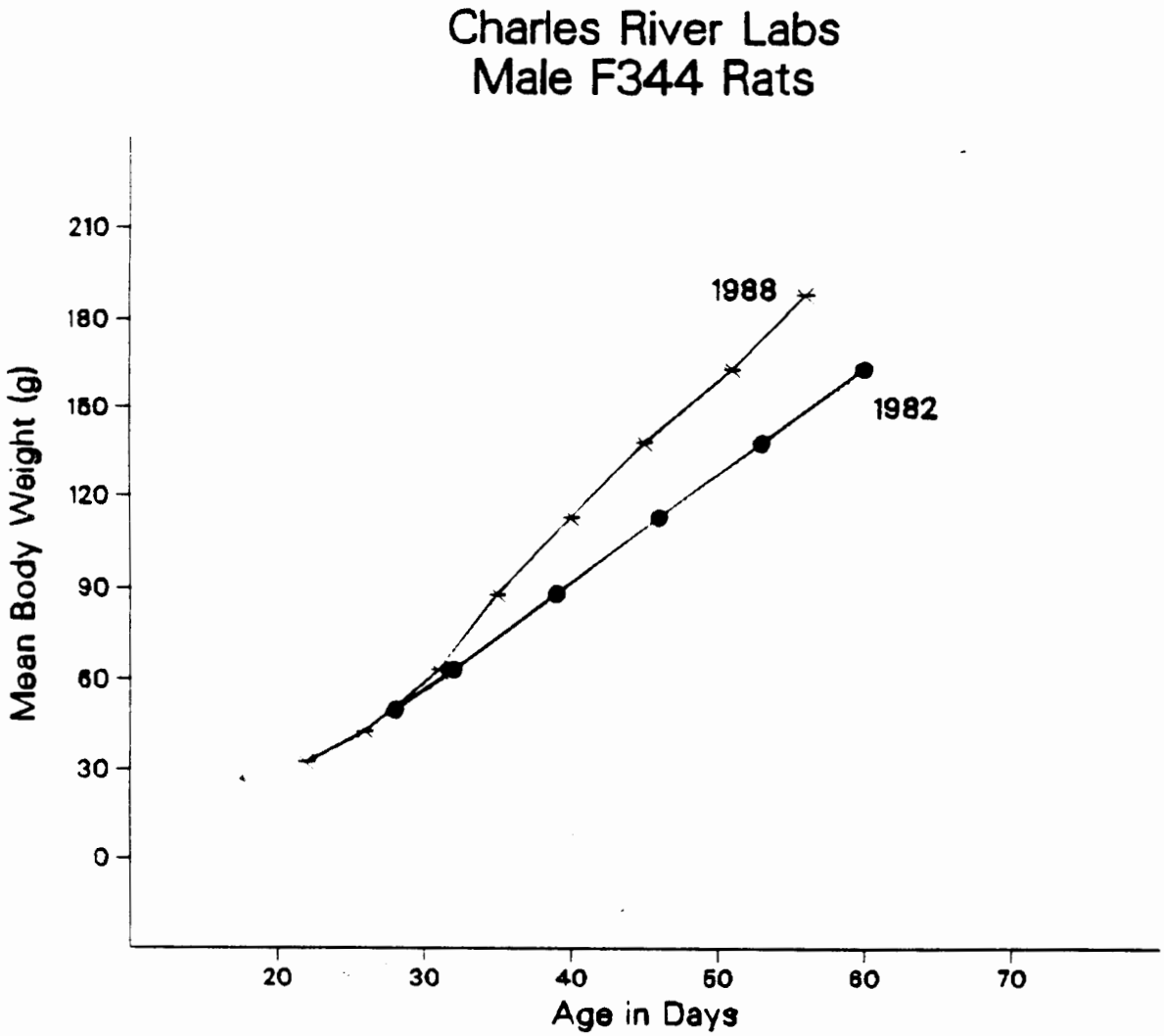

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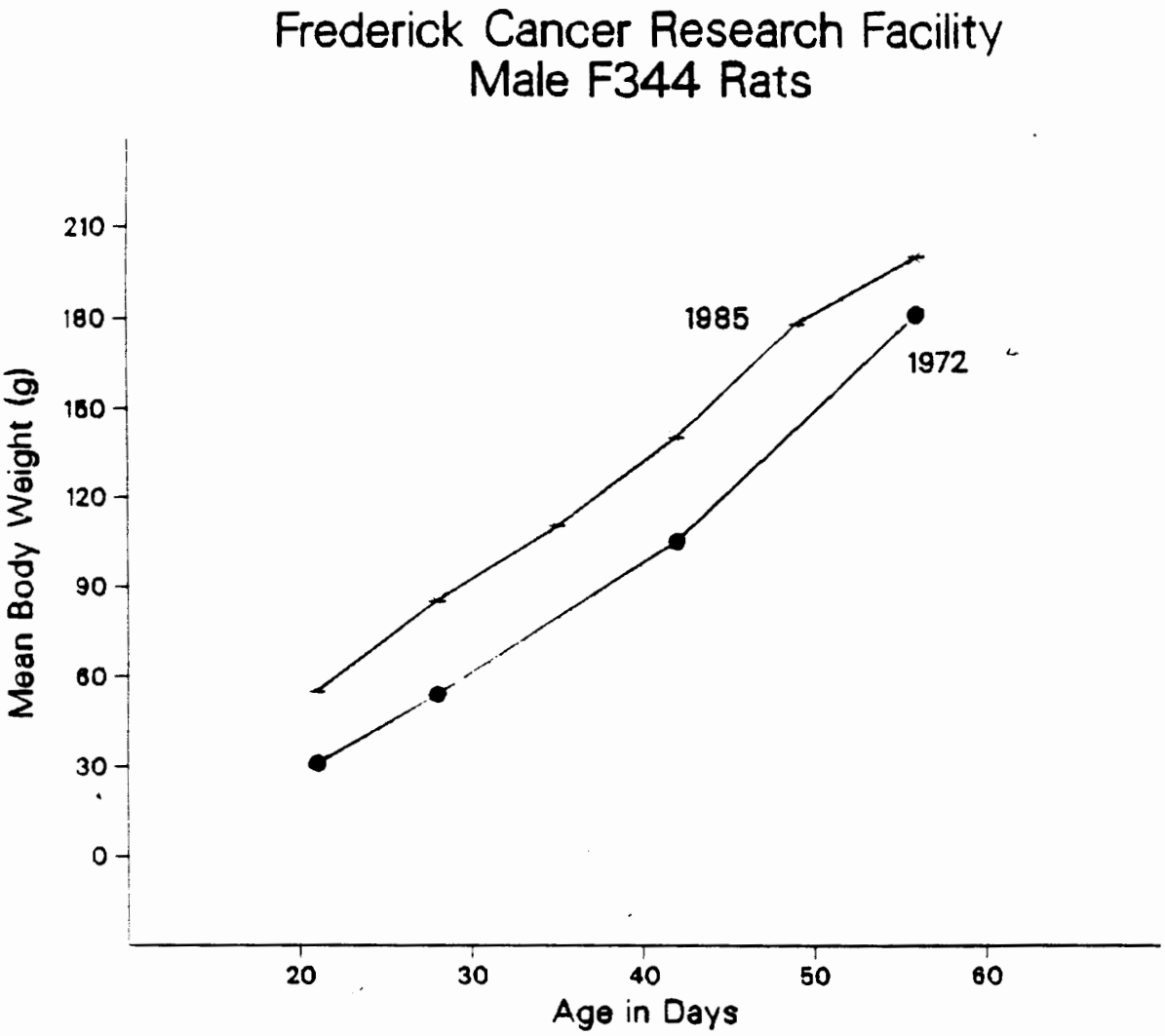

Slide 19 (Figure 16)

Slide 20

INDUSTRY EXPERIENCE WITH F344 RATS

Slide 21 (Figure 17)

Slide 22

INDUSTRY EXPERIENCE WITH SPRAGUE-DAWLEY RATS

Slide 23

FASTER GROWTH AND INCREASING BODY WEIGHT IN RECENT YEARS MAY BE DUE TO

- Elimination of infectious disease.
- Improved diet that is not deficient in essential nutrients but may be high in caloric density and protein.
- Control of environmental conditions to decrease stress and energy loss.
- Rats had >60 generations during the last 20 years. It is equivalent to >1000 years in human terms. There may be subtle genetic changes during these 60 generations.
- Intentional or inadvertent selection of fast growing high body weight progeny for future generations.

Slide 24

CONTRIBUTING FACTORS FOR DECREASING SURVIVAL

- Leukemia
- Body weight by increasing pituitary and mammary tumors
- Nephropathy

Slide 25

(Table II)

NUTRIENT(%)	STANDARD DIET (NIH-07)	MODIFIED DIET (NTP-88)
Protein	23	15
Fat	5.5	3.2
Dietary fiber	8	12
Carbohydrate (other than fiber)	45	52.3
Ash and moisture	18.5	17.5

Slide 26

(Table III)

INFLUENCE OF DIETARY PROTEIN CONCENTRATION ON SEVERITY OF NEPHROPATHY IN MALE F344 RATS

Dietary protein concentration	23%	15%
Severity of nephropathy	3.3(10)*	1.4(10)*
24-hr urine volume (ml/rat)	20.9(5)	14.8(5)
Total urinary protein (mg/dl)	1430(5)	455(5)

* number of samples

Nephropathy grading Minimal-1, Mild-2, Moderate-3, Marked-4

Slide 27 (Histology slide of kidney with marked nephropathy)

Slide 28 (Histology slide of kidney with mild nephropathy)

Slide 29

LEUKEMIA

- Major cause of mortality in F344 rat
- Not related to body weight
- ~40% diet restriction did not decrease the incidence of leukemia
- ~20% diet restriction did not increase survival
- Corn oil gavage decreased the incidence of leukemia eventhough there is an increase in body weight in male F344 rats

Slide 30

(Table IV)

INFLUENCE OF CORN OIL GAVAGE ON BODY WEIGHT AND PREVALENCE OF LEUKEMIA IN FISCHER 344 RATS[a].

Treatment	Number of Studies	Male Rats		Female Rats	
		MMBW[b](g)	Leukemia(%)	MMBW[b](g)	Leukemia(%)
Corn oil gavage control	22	480 ± 21	13.8 ± 8.1	302 ± 18	17.8 ± 8.9
Untreated diet control	37	449 ± 23	26.5 ± 8.8	322 ± 23	17.3 ± 6.0

[a]Numbers are means ± SD
[b]Maximum mean body weight
From Haseman, Huff, Rao, et al., 1985

Slide 31

DIETARY CONSIDERATIONS TO INCREASE SURVIVAL

- Lower the PROTEIN concentration to ~15% to decrease the severity of nephropathy
- Increase the CORN OIL type of fat to decrease the incidence of leukemia (especially in the male F344 rats)
- Decrease the CALORIC DENSITY to lower the body weight by ~15% to decrease the incidences of anterior pituitary tumors and mammary tumors

Slide 32

(Table V)

COMPOSITION OF HIGH FIBER, LOWER PROTEIN AND HIGH CORN OIL DIET (NTP-90)

Nutrient (%)	NIH-07	NTP-90
Protein	23	14.5
Fat	5.5 (soy oil 2.5%)	7.5 (corn oil 5.5%)
Dietary fiber	8	25
Carbohydrate (other than fiber)	45	36
Ash and moisture	18.5	17