NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of β-Picoline (CASRN 108-99-6) in F344/N Rats and B6C3F1/N Mice (Drinking Water Studies)

NTP TR 580

AUGUST 2014

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Technical Report 580

August 2014

National Toxicology Program
Public Health Service
U.S. Department of Health and Human Services
ISSN: 2378-8925

Research Triangle Park, North Carolina, USA

Foreword

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance's carcinogenic potential.

NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

The NTP Technical Reports are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these studies are included in NTP's Chemical Effects in Biological Systems database.

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Explanation of Levels of Evidence of Carcinogenic Activity

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of evidence observed in each experiment: two categories for positive results **(clear evidence and some evidence)**; one category for uncertain findings **(equivocal evidence)**; one category for no observable effects **(no evidence)**; and one category for experiments that cannot be evaluated because of major flaws **(inadequate study)**. These categories of interpretative conclusions were first adopted in June 1983 and then revised on March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been

adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as "were also related" to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as "may have been" related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

Peer Review

The members of the Peer Review Panel who evaluated the draft *NTP Technical Report on the Toxicology and Carcinogenesis Studies of β-Picoline (CASRN 108-99-6) in F344/N Rats and B6C3F1/N Mice* on February 8, 2012, are listed below. Panel members served as independent scientists, not as representatives of any institution, company, or governmental agency.

In this capacity, panel members had five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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Publication Details

Publisher: National Toxicology Program

Publishing Location: Research Triangle Park, NC

ISSN: 2378-8925

DOI: https://doi.org/10.22427/NTP-TR-580

Report Series: NTP Technical Report Series

Report Series Number: 580

Official citation: National Toxicology Program (NTP). 2014. NTP technical report on the toxicology and carcinogenesis studies of β-picoline (CASRN 108-99-6) in F344/N rats and B6C3F1/N mice (drinking water studies). Research Triangle Park, NC: National Toxicology Program. Technical Report 580.

Abstract

β-Picoline is used as a solvent in the synthesis of pharmaceuticals, resins, dyes, rubber accelerators, and insecticides. β-Picoline was nominated by the National Institute of Environmental Health Sciences for toxicological evaluation and carcinogenicity studies based on its high production volume and potential for human exposure. Male and female F344/N rats and B6C3F1/N mice were exposed to β-picoline (greater than 96% pure) in drinking water for 3 months or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

Three-month Study in Rats

Groups of 10 male and 10 female core study rats were exposed to 0, 78, 156, 312, 625, or 1,250 mg β-picoline/L drinking water for 14 weeks (equivalent to average daily doses of approximately 6, 11, 22, 38, or 70 mg β-picoline/kg body weight to males and 6, 12, 23, 38, or 64 mg/kg to females). Special study groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days for determinations of cytochrome P450 enzyme activity. All rats survived to the end of the study. Mean body weights of males and females exposed to 625 or 1,250 mg/L were significantly less than those of the controls. Water consumption by 625 and 1,250 mg/L males and females was less than that by the controls at weeks 1 and 13 due to poor palatability. On day 23, hepatic 7-pentoxyresorufin-*O*-dealkylase activity was significantly increased in 312 mg/L or greater males and in 156 mg/L or greater females compared to that in the controls. Absolute liver weights of 625 and 1,250 mg/L males and absolute and relative liver weights of 625 and 1,250 mg/L females were significantly less than those of the controls. The Markov transition matrix analyses of estrous cyclicity indicated female rats in the 312 and 625 mg/L groups had a significantly higher probability of extended estrus than the control females, suggesting a potential for β-picoline to be a reproductive toxicant in female rats exposed to these concentrations.

The severity of chronic progressive nephropathy was increased in 625 and 1,250 mg/L males and that of hyaline droplet accumulation in proximal renal tubules was increased in 1,250 mg/L males. The concentrations of renal α2u-globulin were significantly increased in 312 mg/L or greater males compared to the controls.

Three-month Study in Mice

Groups of 10 male and 10 female mice were exposed to 0, 78, 156, 312, 625, or 1,250 mg β-picoline/L drinking water for 14 weeks (equivalent to average daily doses of approximately 10, 20, 37, 77, or 148 mg β-picoline/kg body weight to males and 9, 18, 38, 72, or 134 mg/kg to females). All mice survived to the end of the study. Mean body weights and water consumption were generally similar among exposed and control groups of male and female mice. Lung weights of 1,250 mg/L females were significantly less than those of the controls. No histopathologic lesions were attributed to β-picoline exposure.

Two-year Study in Rats

Groups of 50 male and 50 female rats were exposed to 0, 156.25, 312.5, or 625 mg β-picoline/L drinking water for 104 or 105 weeks (equivalent to average daily doses of approximately 6, 12, or 22 mg β-picoline/kg body weight to males and 7, 14, or 26 mg/kg to females). Survival and mean body weights were generally similar among exposed and control groups of male and

female mice. Decreased water consumption was evident in 625 mg/L males and females compared to controls throughout the 2-year study.

The incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in the lung of 625 mg/L female rats.

Two-year Study in Mice

Groups of 50 male and 50 female mice were exposed to 0, 312.5, 625, or 1,250 mg β-picoline/L drinking water for 105 weeks (equivalent to average daily doses of approximately 26, 50, or 92 mg β-picoline/kg body weight to males and 18, 37, or 68 mg/kg to females). Survival of all exposed groups was similar to that of the control groups. Mean body weights of 1,250 mg/L males were 10% less than those of the control group after week 57, and those of 1,250 mg/L females were generally 10% less after week 13. Water consumption by exposed groups of males and females was similar to that by controls during the first 13 weeks of the study; water consumption by 625 and 1,250 mg/L males and 1,250 mg/L females was less than that in the controls after week 13.

In the liver of females, there were significantly increased incidences of hepatocellular adenoma in the 312.5 mg/L group and hepatocellular carcinoma in all exposed groups. The combined incidences of hepatocellular carcinoma or hepatoblastoma were significantly increased in all exposed females.

In the lung, the incidence of alveolar/bronchiolar adenoma in 625 mg/L males was significantly increased. The incidences of alveolar/bronchiolar adenoma occurred with a positive trend in females. The incidences of alveolar/bronchiolar carcinoma were increased in all exposed groups of females. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) was significantly increased in 1,250 mg/L females. The incidence of alveolar epithelium hyperplasia was significantly increased in 1,250 mg/L females.

In the nose, there were significantly increased incidences of olfactory epithelium respiratory metaplasia in 625 mg/L males and 1,250 mg/L males and females; the incidence of olfactory epithelium atrophy was significantly increased in 1,250 mg/L females.

Genetic Toxicology

β-Picoline was tested in three independent bacterial gene mutation studies; all studies gave negative results in *S. typhimurium* or *E. coli* tester strains, with and without exogenous metabolic activation. In vivo, no significant increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female B6C3F1/N mice treated with β-picoline in drinking water for 3 months.

Conclusions

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity* (see Explanation of Levels of Evidence of Carcinogenic Activity; see summary of the peer review panel comments and the public discussion on this Technical Report in Appendix M) of β-picoline in male F344/N rats exposed to 156.25, 312.5, or 625 mg/L. There was *some evidence of carcinogenic activity* of β-picoline in female F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of β-picoline in male B6C3F1/N mice based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *clear evidence of*

carcinogenic activity of β-picoline in female B6C3F1/N mice based on the increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in the lung and of hepatocellular carcinoma and hepatoblastoma in the liver.

Exposure to β-picoline caused increased incidences of nonneoplastic lesions of the lung in female mice and the nose in male and female mice.

Synonyms: β-methylpyridine; 3-methylpyridine; m-methylpyridine; m-picoline

Summary of the Two-year Carcinogenesis and Genetic Toxicology Studies of β-Picoline

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in drinking water	0, 156.25, 312.5, or 625 mg/L	0, 156.25, 312.5, or 625 mg/L	0, 312.5, 625, or 1,250 mg/L	0, 312.5, 625, or 1,250 mg/L
Body weights	Exposed groups generally similar to the control group	Exposed groups similar to the control group	1,250 mg/L group 10% less than the control group after week 57	1,250 mg/L group generally 10% less than the control group after week 13
Survival rates	33/50, 31/50, 32/50, 24/50	30/50, 32/50, 33/50, 30/50	24/50, 26/50, 27/50, 33/50	38/50, 32/50, 35/50, 33/50
Nonneoplastic effects	None	None	Nose: olfactory epithelium, metaplasia, respiratory (8/50, 12/50, 30/50, 41/50)	Lung: alveolar epithelium, hyperplasia (2/50, 4/50, 3/49, 8/50) Nose: olfactory epithelium, metaplasia, respiratory (2/49, 2/44, 7/49, 14/47); olfactory epithelium, atrophy (1/49, 2/44, 2/49, 7/47)
Neoplastic effects	None	Lung: alveolar/bronchiolar adenoma (0/50, 3/50, 2/50, 5/50); alveolar/bronchiolar adenoma or carcinoma (0/50, 4/50, 2/50, 5/50)	None	Liver: hepatocellular carcinoma (11/49, 20/50, 26/50, 23/50); hepatoblastoma (1/49, 3/50, 4/50, 4/50) Lung: alveolar/bronchiolar adenoma (5/50, 6/50, 4/49, 11/50); carcinoma (7/50, 8/50, 10/49, 13/50); alveolar/bronchiolar adenoma or carcinoma (11/50, 13/50, 13/49, 21/50)
Equivocal findings	None	None	Lung: alveolar/bronchiolar adenoma (6/50, 11/50, 16/50, 8/50); alveolar/bronchiolar adenoma or carcinoma (14/50, 19/50, 21/50, 15/50)	None
Level of evidence of carcinogenic activity	No evidence	Some evidence	Equivocal evidence	Clear evidence
Genetic toxicology				
Bacterial gene mutations:			Negative in *S. typhimurium* strains TA97, TA98, TA100, TA1535, and TA1537 with and without S9; negative in *E. coli* strain WP2 *uvrA*/pKM101 with and without S9	
Micronucleated erythrocytes				
Mouse peripheral blood in vivo:			Negative in males and females	

Introduction

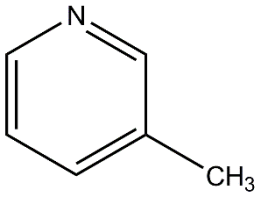

Figure 1. β-Picoline (CASRN 108-99-6; Chemical Formula: C_6H_7N; Molecular Weight: 93.13)

Synonyms: β-methylpyridine; 3-methylpyridine; *m*-methylpyridine; *m*-picoline.

Chemical and Physical Properties

β-picoline is an aromatic heterocyclic compound that is structurally similar to pyridine. It is a clear, pale-yellow liquid with a boiling point of 143 to 144°C and a specific gravity of 0.9613[1]. It is combustible, has a vapor pressure of 6.05 mm Hg at 25°C[2] and a flash point of 40°C[3]. It is very soluble in acetone and miscible with water, alcohol, and ether[1; 2].

Production, Use, and Human Exposure

β-picoline is a high production volume chemical with a reported annual United States production volume of approximately 21.2 to 29.1 million pounds[4]. It is produced from the vapor-phase reaction of acetaldehyde and ammonia with formaldehyde and/or methanol in the presence of a catalyst, or from the vapor-phase reaction of acrolein with ammonia in the presence of an acid catalyst[2]. It can also be produced using cyclohexylamine, ammonia, and zinc chloride or from the pyrolysis of coal tar or bone oil[5].

β-Picoline is used as a solvent in the synthesis of pharmaceuticals, resins, dyes, rubber accelerators, and insecticides[6; 7]. It is also used as a laboratory reagent and as an intermediate in the manufacture of waterproofing agents, niacin, and niacinamide[1; 7]. While occupational exposures primarily occur via inhalation or dermal contact during the production or utilization of β-picoline, environmental exposures to humans can occur via contaminated drinking water and food, ambient air, and dermal contact with products containing β-picoline[2]. Environmental contamination primarily occurs through the release in various waste streams from its use as a solvent, chemical intermediate, and laboratory agent. It is also present in effluents from the manufacture and use of coal-derived liquid fuels and from the disposal of coal liquefaction and gasoline waste byproducts[2]. β-Picoline released into water may volatize into air where it exists as a vapor but remains in water until degraded as it is not expected to adsorb to suspended solids or sediment[2]. β-Picoline is also found as a component of cigarette smoke[2].

Regulatory Status

β-Picoline is regulated by the U.S. Environmental Protection Agency (USEPA) under the Clean Air Act as part of the national emission standards for organic hazardous air pollutants[8]. Additionally, the USEPA regulates β-picoline as a substituted pyridine under the Toxic Substances Control Act by requiring any significant new use to be reported to the USEPA[9], and by requiring manufacturers, importers, and processors to submit unpublished health and safety

studies[10]. The U.S. Coast Guard and the U.S. Department of Transportation (DOT) regulate the transport of β-picoline. The U.S. Coast Guard considers β-picoline a hazardous aromatic amine and requires transport on ocean vessels with other aromatic amines to avoid incompatibility or reactivity with other chemicals[11]. The DOT considers β-picoline a hazardous material and requires specific criteria with respect to labeling and packaging for transport[12].

Absorption, Distribution, Metabolism, and Excretion

There is limited information available on the absorption, distribution, metabolism, and excretion of β-picoline. β-Picoline is readily absorbed from the gastrointestinal tract, intraperitoneal cavity, and the lungs and moderately well absorbed through the skin[3]. Gorrod and Damani[13] investigated in vitro carbon (C) and nitrogen (N)-oxidation of β-picoline to 3-pyridylcarbinol and 3-methylpyridine N-oxide, respectively, with hepatic and lung microsomes from various species. These studies demonstrated species- and sex-specific variability in hepatic N-oxidation with male rats producing greater amounts of 3-methylpyridine N-oxide and 3-pyridylcarbinol than females. The reverse was observed in mice with females producing greater amounts of C- and N-oxidation products than males. Further studies by Gorrod and Damani[14] investigated metabolic N-oxidation of β-picoline in vivo following intraperitoneal administration in mice, hamsters, rats, guinea pigs, rabbits, and ferrets. These studies demonstrated that less than 7% of β-picoline was excreted as 3-methylpyridine N-oxide in the urine of each species.

Toxicity

Experimental Animals

Acute studies with β-picoline have reported an oral LD_{50} in rats and mice of 400 to 1,600 mg/kg, and an intraperitoneal LD_{50} of 100 to 200 mg/kg in rats and 400 to 800 mg/kg in mice. In Long-Evans hooded rats administered 100 mg/kg, Dyer et al.[15] investigated the neurophysiological effects of acute exposure to β-picoline. These studies demonstrated increased latency of evoked potentials and increased latency to pentylenetetrazol-induced seizures, measures of sensory function and cerebral excitability, respectively. These effects are consistent with other chemicals that induce central nervous system depression. In male New Zealand albino rabbits, β-picoline produced moderate to severe dermal and ocular irritation[16]. Histological examination demonstrated keratitis lesions in corneal epithelial tissue, fibrillary edematous lamellae dissociation, and cellular inflammatory infiltration in the eye, and necrosis, ulceration, and regeneration in the skin.

β-Picoline is structurally related to pyridine, but differs from pyridine in the presence of a methyl group on the beta (3-) carbon. The National Toxicology Program (NTP) has previously conducted toxicology and carcinogenesis studies of pyridine administered in drinking water[17]. Thirteen-week and 2-year studies were conducted in male and female F344/N rats and B6C3F1 mice, and male Wistar rats. In the 13-week subchronic studies, pyridine administration in the drinking water induced significant liver toxicity and/or altered hepatic function in both F344/N and Wistar rats. Kidney lesions consistent with α2u-globulin nephropathy were observed in F344/N males, but these lesions were not observed in the Wistar males, which are generally considered nonresponsive to chemicals that mediate α2u-globulin nephropathy. In mice, no treatment-related lesions were observed after 13 weeks of exposure.

Humans

No specific toxicity studies of β-picoline in humans were found in the literature.

Reproductive and Developmental Toxicity

No information regarding the reproductive and developmental effects of β-picoline in experimental animals or humans were found in the literature.

Carcinogenicity

No information regarding the carcinogenic effects of β-picoline in experimental animals or epidemiology studies in humans were found in the literature. In the National Toxicology Program[17] carcinogenicity studies, administration of pyridine induced renal tubule neoplasms in male F344/N rats and malignant hepatocellular neoplasms in male and female B6C3F1 mice. There were also increased incidences of mononuclear cell leukemia in male F344/N rats and interstitial cell adenoma of the testis in male Wistar rats. Based on these findings, it was concluded that there was evidence of carcinogenic activity in male F344/N rats and male and female B6C3F1 mice.

Genetic Toxicity

There is no evidence that β-picoline induces genetic damage, although published studies are limited to three bacterial gene mutation assays. β-Picoline did not induce revertants, in tests conducted with or without metabolic activation, in *Salmonella typhimurium* strains TA97, TA98, TA100, or TA102 at concentrations up to 5,000 µg/plate[18] or in strains TA98, TA100, TA1535, or TA1537 at concentrations ranging up to 8,540 µg/plate[19]. Ho et al.[20] also reported negative results for gene mutation induction in *S. typhimurium* strain TA98 tested with β-picoline concentrations up to 1,000 µg/plate.

Study Rationale

β-Picoline was nominated by the National Institute of Environmental Health Sciences for toxicological evaluation and carcinogenicity studies based on its high production volume and potential for human exposure. Animals were exposed via the drinking water because this is a primary route of exposure for humans.

Materials and Methods

Procurement and Characterization

β-Picoline

β-Picoline was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), in one lot (11108CI) that was used during the 3-month and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Chemistry Support Services (Columbus, OH) and the study laboratory at Battelle Columbus Operations (Columbus, OH); Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN) (Appendix I). One additional lot (14517PS) obtained from Aldrich Chemical Company was used by the analytical chemistry laboratory for dose formulation stability studies and was not used in the 3-month or 2-year animal studies. Reports on analyses performed in support of the β-picoline studies are on file at the National Institute of Environmental Health Sciences.

Lot 11108CI, a clear, pale-yellow liquid, was identified as β-picoline by the analytical chemistry laboratory and the study laboratory using infrared spectroscopy; the analytical chemistry laboratory also used proton and carbon-13 nuclear magnetic resonance spectroscopy. Karl Fischer titration was used to determine the water content of lot 11108CI and elemental analyses were used to determine the carbon, hydrogen, and nitrogen content. The purity of lot 11108CI was determined by the analytical chemistry laboratory using gas chromatography and differential scanning calorimetry.

Differential scanning calorimetry analysis indicated a purity of 96.4%. Karl Fischer titration indicated a 2.5% water content. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for β-picoline. Gas chromatography with flame ionization detection analysis indicated one major peak (β-picoline) and only two impurities with individual peak areas greater than 0.1%. The peak areas for the impurities represented a total of 1% of lot 11108CI (0.6% and 0.4%, respectively).

To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles sealed with Teflon®-lined lids under a nitrogen head space.

Periodic reanalyses of the bulk chemical were performed by the study laboratory using gas chromatography at the beginning and end of the 3-month and 2-year studies, and approximately every 6 months during the 2-year studies; no degradation of the bulk chemical occurred.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared five times for the 3-month studies and approximately every 4 weeks for the 2-year studies by mixing β-picoline with tap water (Table I-1). The pH was adjusted if necessary to bring it within the range of 6 to 7.5 by the addition of acetic acid. The dose formulations were determined to be true solutions. Stability studies of 10 µg/mL formulations were performed by the analytical chemistry laboratory using a high-performance liquid chromatography with ultraviolet light detection. Stability was confirmed for at least 42

days for formulations stored in sealed polyethylene bottles protected from light at 5°C and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of β-picoline were performed by the study laboratory using high-performance liquid chromatography with ultraviolet detection. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations analyzed and used for rats and mice were within 10% of the target concentrations (Table I-2). Animal room samples of these dose formulations were also analyzed; all 15 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2 year studies, the dose formulations were analyzed at least every 12 weeks. All 50 dose formulations analyzed and used for rats and all 50 for mice were within 10% of the target concentrations (Table I-3). Animal room samples were also analyzed; all 12 animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

Three-month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to β-picoline and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc., Germantown, NY. On receipt, the rats and mice were approximately 4 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and mice for 14 (females) or 15 (males) days. Rats were 5 to 6 weeks old and mice were 6 to 7 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 1 month and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female core study rats and mice were exposed to 0, 78, 156, 312, 625, or 1,250 mg β-picoline/L drinking water for 14 weeks. Special study groups of 10 male and 10 female rats were exposed to the same concentrations for 23 days. Feed and water were available ad libitum. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings for core study rats and mice were recorded once a week beginning on day 1 and at the end of the studies. Water consumption was recorded weekly by cage. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Table 1. Experimental Design and Materials and Methods in the Drinking Water Studies of β-Picoline

Three-month Studies	Two-year Studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/N rats B6C3F1/N mice	F344/N rats B6C3F1/N mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies	
Rats: 11 (males) or 12 (females) days Mice: 15 (males) or 14 (females) days	Rats: 14 (males) or 15 (females) days Mice: 12 (males) or 11 (females) days
Average Age When Studies Began	
Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 6 to 7 weeks Mice: 5 to 6 weeks
Date of First Exposure	
Rats: November 17 (males) or 18 (females), 2003 Mice: November 21 (males) or 20 females), 2003	Rats: November 18 (males) or 19 (females), 2004 Mice: November 9 (males) or 8 (females), 2004
Duration of Exposure	
Core studies: 14 weeks Special study rats: 4 weeks	104 (male rats) or 105 weeks
Date of Last Exposure	
Rats: Core study: February 17 (males) or 18 (females), 2004 Special study: December 9 (males) or 10 (females), 2003 Mice: February 20 (males) or 19 (females), 2004	Rats: November 15 (males) or 17 (females), 2006 Mice: November 10 (males) or 8 (females), 2006
Necropsy Dates	
Rats: February 17 (males) or 18 (females), 2004 Mice: February 20 (males) or 19 (females), 2004	Rats: November 13–15 (males) or 15–17 (females), 2006 Mice: November 8–10 (males) or 6–8 (females), 2006
Average Age at Necropsy	
19 to 20 weeks	Rats: 110 or 111 weeks Mice: 110 or 111 weeks (males); 109 or 110 weeks (females)
Size of Study Groups	
10 males and 10 females	50 males and 50 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-month studies

Three-month Studies	Two-year Studies
Animals per Cage	
Rats: 5 Mice: 1 (males) or 5 (females)	Rats: 2 or 3 (males) or 5 (females) Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
Irradiated NTP-2000 wafer feed (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed at least weekly	Same as 3-month studies
Water	
Tap water (Columbus municipal supply) via glass sipper tube water bottles (glass bottles: Supelco, Inc., Bellfonte, PA; stainless steel double-ball bearing sipper tubes: Ancare Corp., Bellmore, NY), available ad libitum, changed at least twice weekly	Same as 3-month studies, except glass bottles used for rats and female mice supplied by VWR (West Chester, PA)
Cages	
Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly (male mice) or twice weekly (rats and female mice) and rotated every 2 weeks	Same as 3-month studies, except changed twice weekly and rotated every 2 weeks
Bedding	
Irradiated Sani-Chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly (male mice) or twice weekly (rats and female mice)	Same as 3-month studies, except changed twice weekly
Rack Filters	
Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 3-month studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed and rotated every 2 weeks	Same as 3-month studies
Animal Room Environment	
Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations	
0, 78, 156, 312, 625, or 1,250 mg/L in drinking water, available ad libitum	Rats: 0, 156.25, 312.5, or 625 mg/L in drinking water, available ad libitum Mice: 0, 312.5, 625, or 1,250 mg/L in drinking water, available ad libitum

Three-month Studies	Two-year Studies
Type and Frequency of Observation	
Observed twice daily; clinical findings were recorded and core study animals were weighed initially, weekly, and at the end of the studies. Water consumption was recorded weekly by cage.	Observed twice daily; clinical findings were recorded every 4 weeks beginning week 5 and at the end of the studies; animals were weighed initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies. Water consumption was recorded weekly for the first 13 weeks of the study, then monthly thereafter by cage.
Method of Kill	
Carbon dioxide asphyxiation	Carbon dioxide asphyxiation
Necropsy	
Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology	
Blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only). Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials Clinical chemistry: urea nitrogen, creatinine, glucose (week 14 only), total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Cytochrome P450 Activity	
On day 23, tissue samples from the liver of special study rats were taken for determination of 7-pentoxyresorufin-*O*-dealkylase activity.	None
Histopathology	
Complete histopathologic examinations were performed on 0 and 1,250 mg/L core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), left kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The left kidney was also examined in the remaining groups of core study rats.	Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice only), Harderian gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, oral mucosa, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, tongue, trachea, urinary bladder, and uterus.

Three-month Studies	Two-year Studies
α2u-Globulin	
At the end of the study, the right kidneys of core study male rats were collected for α2u-globulin determination.	None
Sperm Motility and Vaginal Cytology	
At the end of the studies, spermatid and sperm samples were collected from male rats in the 0, 156, 312, and 625 mg/L groups and from male mice in the 0, 312, 625, and 1,250 mg/L groups. The following parameters were evaluated: spermatid heads per testis and per gram testis, sperm motility, and sperm per cauda epididymis and per gram cauda epididymis. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female rats in the 0, 156, 312, and 625 mg/L groups and from female mice in the 0, 312, 625, and 1,250 mg/L groups.	None

For determination of hepatic cytochrome P450 2B1 (CYP2B1) activity, liver tissue samples were collected from special study rats on day 23 and stored frozen at −70°C until shipped to Battelle Toxicology Northwest (Richland, WA) for analysis. Microsomal suspensions were prepared using the Pearce Method[21]. The concentration of protein in each suspension was determined using the microtiter plate method of the Coomassie® Plus Protein Assay (Pierce Chemical Co., Rockford, IL) with bovine serum albumin as the standard. CYP2B1-associated pentoxyresorufin-*O*-dealkylase activities were determined in microsomal proteins and isolated from frozen liver samples according to established procedures.

After anesthesia with carbon dioxide, blood was collected from the retroorbital sinus of special study rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only) analyses. Blood samples for hematology analyses were placed in tubes containing EDTA and gently inverted to prevent clotting. All automated hematology measurements were performed on an ADVIA® 120 Hematology Analyzer (Bayer, Inc., Tarrytown, NY) using reagents supplied by manufacturer (Siemens Healthcare Diagnostics, city, state). Leukocyte differentials were counted on slides stained with a modified Wright's stain using an Ames Hema-Tek slide stainer (Miles Laboratory, Ames Division, Elkhart, IN). Leukocyte and reticulocyte counts, erythrocyte and platelet counts and morphologies were determined from blood smears by light microscopy. Clinical chemistry parameters in rats were determined using a Roche Hitachi 911 chemistry analyzer (Roche Diagnostics, Corp., Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on core study rats exposed to 0, 156, 312, or 625 mg/L and mice exposed to 0, 312, 625, or 1,250 mg/L. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal kill, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were collected and stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left

epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (except eyes were first fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin; the left kidney of rats was also stained with Mallory Heidenhain. Complete histopathologic examinations were performed on 0 and 1,250 mg/L core study rats and mice; the left kidney was examined in all core study rats. Table 1 lists the tissues and organs routinely examined.

After weighing, the right kidneys of core study rats were frozen and shipped to Battelle Toxicology Northwest for measurement of soluble protein and α2u-globulin concentrations. Kidneys were thawed, homogenized with sodium/potassium phosphate buffer (pH 7.2), and centrifuged. Soluble protein concentrations in the supernatants were determined using the Roche (biuret) Total Protein Assay (Roche Diagnostics Corp.) performed on the Hitachi 912 Analyzer (Roche Diagnostics Corp.). Analyses of α2u-globulin were conducted using the Quantikine® M Rat α2u-Globulin Immunoassay (R&D Systems, Inc., Minneapolis, MN).

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[22] and Boorman et al.[23].

Two-year Studies

Study Design

Groups of 50 male and 50 female rats were exposed to 0, 156.25, 312.5, or 625 mg β-picoline/L drinking water for 104 (males) or 105 (females) weeks. Groups of 50 male and 50 female mice were exposed to drinking water containing β-picoline at concentrations of 0, 312.5, 625, or 1,250 mg/L for 105 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1/N mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats were quarantined for 14 or 15 days and mice for 11 or 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 6 to 7 weeks old and mice 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program using extra animals at 1 month; sentinel animals at 6, 12, and 18 months; and 625 mg/L rats and 1,250 mg/L mice at the end of the studies; five males and five females were used at each timepoint (Appendix L).

Animal Maintenance

All animal studies were conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. Studies were approved by the Battelle Columbus Operations Animal Care and Use Committee and conducted in accordance with all relevant NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Male rats were housed two or three per cage, female rats and mice five per cage, and male mice individually. Feed and water were available ad libitum. Cages were changed twice weekly and rotated every 2 weeks; racks were changed and rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded every 4 weeks beginning week 5 and at study termination. Body weights were recorded initially, weekly for 13 weeks, monthly thereafter, and at study termination. Water consumption was recorded weekly by cage for the first 13 weeks of the study, then at 4-week intervals until the end of the study.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. An examination was conducted for gross lesions of the tongue and remnants of oral cavity for all rats and mice. The tongue of all male and female rats and mice and all untrimmed potential oral mucosa lesions were processed and evaluated. Previously recorded proliferative lesions of the oral cavity were also reevaluated. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The report, slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual

animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the lung of all animals and the liver and nose of mice.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman[22] and Boorman et al.[23]. For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al.[24].

Statistical Methods

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier[25] and is presented in the form of graphs. Animals found dead of other than natural causes were censored; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's[26] method for testing two groups for equality and Tarone's[27] life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Table A-1, Table A-4, Table B-1, Table B-4, Table C-1, Table C-4, Table D-1, and Table D-5 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Table A-2, Table B-2, Table C-2, and Table D-2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Table A-2, Table B-2, Table C-2, and Table D-2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm,

proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal kill.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test[28-30] was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal kill; if the animal died prior to terminal kill and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time[28]. Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier[28] following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice[31]. Bailer and Portier[28] showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams[32].

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P = 0.99 is presented as P = 0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett[33] and Williams[34; 35]. Hematology, clinical chemistry, CYP2B1, α2u-globulin, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[36] (as modified by Williams[37]) and Dunn[38]. Jonckheere's test[39] was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey[40] were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each dosed group were compared to the control group using the Fisher exact test[41]. Tests for extended periods of estrus, diestrus, metestrus, and proestrus, as well as skipped estrus and skipped diestrus, were constructed based on a Markov chain model proposed by Girard and

Sager[42]. For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provisions for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, NTP incorporated a new diet[17] that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies[43; 44]. The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[45]. In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of β-picoline was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division[46; 47]. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by NTP to develop a comprehensive data-base permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term in vitro and in vivo genetic toxicity tests (structure-activity relationships). The short-term tests were originally developed to clarify

proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity[48] and the somatic mutation theory of cancer[49; 50]. However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with Salmonella mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites[51]. A positive response in the Salmonella test was shown to be the most predictive in vitro indicator for rodent carcinogenicity (89% of the Salmonella mutagens are rodent carcinogens)[52; 53]. Additionally, no battery of tests that included the Salmonella test improved the predictivity of the Salmonella test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute in vivo bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test[54; 55]. However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies[56]. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of in vivo genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical.

Results

Rats

Three-month Study

All rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of males and females exposed to 625 or 1,250 mg/L were significantly less than those of the controls (Table 2 and Figure 2). Water consumption by 625 and 1,250 mg/L males and females was less than that by the controls at weeks 1 and 13; this was attributed to unpalatability of the chemical at these concentrations. Drinking water concentrations of 78, 156, 312, 625, and 1,250 mg/L resulted in average daily doses of approximately 6, 11, 22, 38, and 70 mg β-picoline/kg body weight to males and 6, 12, 23, 38, and 64 mg/kg to females. There were no clinical findings related to exposure to β-picoline.

Table 2. Survival, Body Weights, and Water Consumption of Rats in the Three-month Drinking Water Study of β-Picoline[a]

Concentration (mg/L)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Water Consumption Week 1	Water Consumption Week 13
Male							
0	10/10	101 ± 3	356 ± 5	255 ± 4		14.3	16.1
78	10/10	101 ± 3	349 ± 7	248 ± 5	98	13.8	14.8
156	10/10	101 ± 2	352 ± 4	251 ± 4	99	12.9	15.4
312	10/10	103 ± 3	356 ± 4	253 ± 4	100	14.1	14.7
625	10/10	100 ± 3	332 ± 4**	231 ± 5**	93	10.2	11.4
1,250	10/10	102 ± 3	298 ± 5**	196 ± 4**	83	6.7	12.1
Female							
0	10/10	95 ± 2	207 ± 5	112 ± 4		11.8	10.7
78	10/10	97 ± 2	206 ± 2	109 ± 3	100	12.5	9.3
156	10/10	97 ± 2	205 ± 4	109 ± 3	99	12.3	10.5
312	10/10	98 ± 2	205 ± 3	105 ± 3	99	11.5	10.5
625	10/10	94 ± 2	192 ± 3**	98 ± 2**	93	7.9	8.8
1,250	10/10	94 ± 3	177 ± 4**	83 ± 3**	86	6.5	6.7

**Significantly different ($P \leq 0.01$) from the control group by Williams' test.
[a]Weights and weight changes are given as mean ± standard error. Water consumption is expressed as grams per animal per day.
[b]Number of animals surviving at 14 weeks/number initially in group.

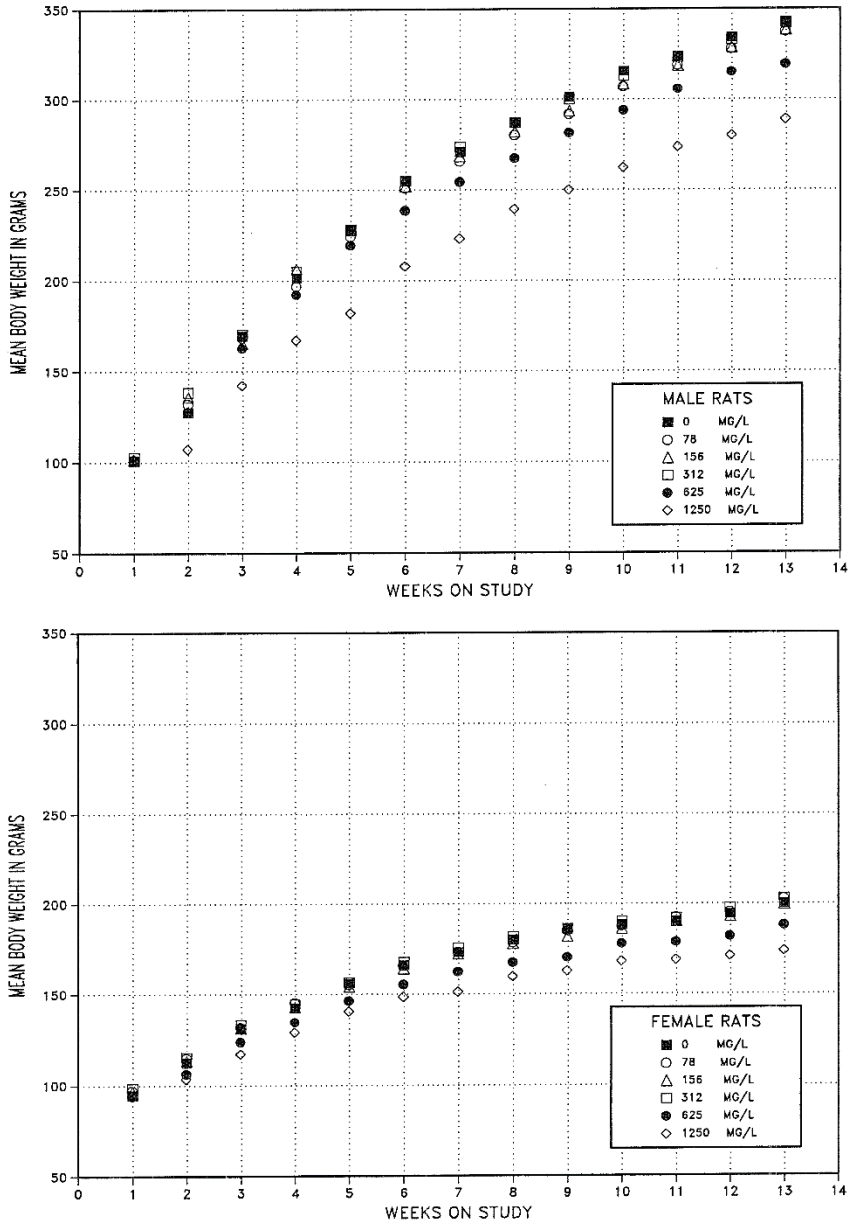

Figure 2. Growth Curves for Rats Exposed to β-Picoline in Drinking Water for Three Months

Pyridine, which is structurally similar to β-picoline, induces CYP2B1 protein and mRNA levels in cultured hepatocytes and rat liver[57-59]. To investigate the effects of β-picoline on hepatic CYP 2B1, liver samples were collected on day 23 from special study rats. Microsomal suspensions were prepared from the liver samples and were assayed for 7-pentoxyresorufin-*O*-dealkylase (PROD) activity (a marker for CYP2B1 activity). Hepatic PROD activity was significantly increased in 312 mg/L or greater males and in 156 mg/L or greater females compared to that in the controls (Table 3). These findings indicate a dose-dependent hepatic enzyme induction in these groups.

Table 3. Hepatic 7-Pentoxyresorufin-*O*-dealkylase (PROD) Activity in Special Study Rats Administered β-Picoline for 23 Days in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
n	10	10	10	10	10	10
Male						
PROD[b]	3.736 ± 0.129	3.798 ± 0.153	3.891 ± 0.120	6.839 ± 0.674**	10.815 ± 0.495**	30.095 ± 5.443**
Female						
PROD	4.377 ± 0.129	4.476 ± 0.126	5.640 ± 0.226**	8.365 ± 0.632**	12.022 ± 0.492**	15.450 ± 0.880**

**Significantly different ($P \leq 0.01$) from the control group by Shirley's test.
[a]Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[b]pmol/minute per mg microsomal protein.

The hematology and clinical chemistry data for rats are presented in Table F-1, and there were minimal transient increases in hematocrit values, erythrocyte counts, and hemoglobin, urea nitrogen, albumin, and total protein concentrations in 1,250 mg/L male rats on day 4. The transient increases in these variables would be consistent with a reduced circulating plasma volume (i.e., dehydration) and a minor hemoconcentration. This transient effect was supported by the decreased water consumption noted at week 1. On day 23 and at week 13 there were minor decreases in serum albumin and total protein concentrations (in most instances less than 5%). Significant decreases in serum albumin concentrations were only noted in the 625 and 1,250 mg/L males at week 13; females were not affected. The potential significance of the minimal decreases was unknown but may be physiologically related to an altered nutritional status as suggested by the substantially decreased body weights.

Absolute liver weights of 625 and 1,250 mg/L males and absolute and relative liver weights of 625 and 1,250 mg/L females were significantly less than those of the controls (Table G-1). Other organ weight differences were related to reduced body weights.

There were no significant differences in sperm parameters of male rats exposed to 156, 312, or 625 mg/L when compared to the controls (Table H-1). Exposure-related decreases in testis weights were minimal (<10%) and accompanied parallel decreases in terminal body weights. The Markov transition matrix analyses of estrous cyclicity indicated female rats in the 312 and 625 mg/L groups had a significantly higher probability of extended estrus than the control females (Table H-2 and Table H-3; Figure H-1).

The severity of chronic progressive nephropathy was increased in 625 and 1,250 mg/L males and that of hyaline droplet accumulation in proximal renal tubules was increased in 1,250 mg/L males (Table 4). The concentrations of renal α2u-globulin were significantly increased in 312 mg/L or greater males compared to the controls (Table 4). These findings are consistent with α2u-globulin nephropathy.

Table 4. Incidences of Nonneoplastic Kidney Lesions and α2u-Globulin Concentrations for Male Rats in the Three-month Drinking Water Study of β-Picoline[a]

0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Number Examined Microscopically					
10	10	10	10	10	10
Nephropathy[b]					
10 (1.2)[c]	10 (1.0)	10 (1.0)	10 (1.2)	10 (2.2)	10 (2.9)
Renal Tubule, Accumulation, Hyaline Droplet					
10 (1.3)	10 (1.1)	10 (1.2)	10 (1.1)	10 (1.0)	10 (2.0)
α2u-Globulin Concentration (ng/mL)					
2.044 ± 0.157	1.943 ± 0.113	1.893 ± 0.090	8.676 ± 0.478**	9.955 ± 0.542**	10.124 ± 0.458**
α2u-Globulin (ng/μg soluble protein)					
42.74 ± 2.98	39.39 ± 2.37	38.76 ± 1.56	181.92 ± 9.62**	214.52 ± 7.04**	217.60 ± 11.16**
α2u-Globulin (nmol/g kidney)					
174.9 ± 13.4	166.2 ± 9.6	161.9 ± 7.7	742.3 ± 40.9**	851.8 ± 46.3**	866.2 ± 39.2**
α2u-Globulin (mg/g kidney)					
3.280 ± 0.249	3.110 ± 0.183	3.040 ± 0.140	13.910 ± 0.766**	15.970 ± 0.864**	16.210 ± 0.734**

**Significantly different ($P \leq 0.01$) from the control group by Shirley's test.
[a]α2u-Globulin data are given as mean ± standard error, n = 10. Statistical tests were performed on unrounded data.
[b]Number of animals with lesion.
[c]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Exposure Concentration Selection Rationale: Based on decreased mean body weights (16% and 14% in males and females, respectively), decreased water consumption in both sexes, and the severity of nephropathy observed in males at 1,250 mg/L, β-picoline exposure concentrations selected for the 2-year drinking water study in rats were 156.25, 312.5, and 625 mg/L.

Two-year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 3). Survival of exposed groups of male and female rats was similar to that of the control groups.

Table 5. Survival of Rats in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Male				
Animals initially in study	50	50	50	50
Moribund	9	12	15	23
Natural deaths	8	7	3	3
Animals surviving to study termination	33	31	32	24
Percent probability of survival at end of study[a]	66	62	64	48
Mean survival (days)[b]	668	700	697	681
Survival analysis[c]	P = 0.092	P = 1.000	P = 1.000	P = 0.160
Female				
Animals initially in study	50	50	50	50
Moribund	15	13	14	15
Natural deaths	5	5	3	5
Animals surviving to study termination	30[d]	32	33	30
Percent probability of survival at end of study	60	64	66	60
Mean survival (days)	688	688	700	686
Survival analysis	P = 0.951	P = 0.853N	P = 0.671N	P = 1.000

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]The result of the life table trend test[27] is in the control column, and the results of the life table pairwise comparisons[26] with the controls are in the exposed group columns. A lower mortality in an exposure group is indicated by **N**.
[d]Includes one animal that died during the last week of the study.

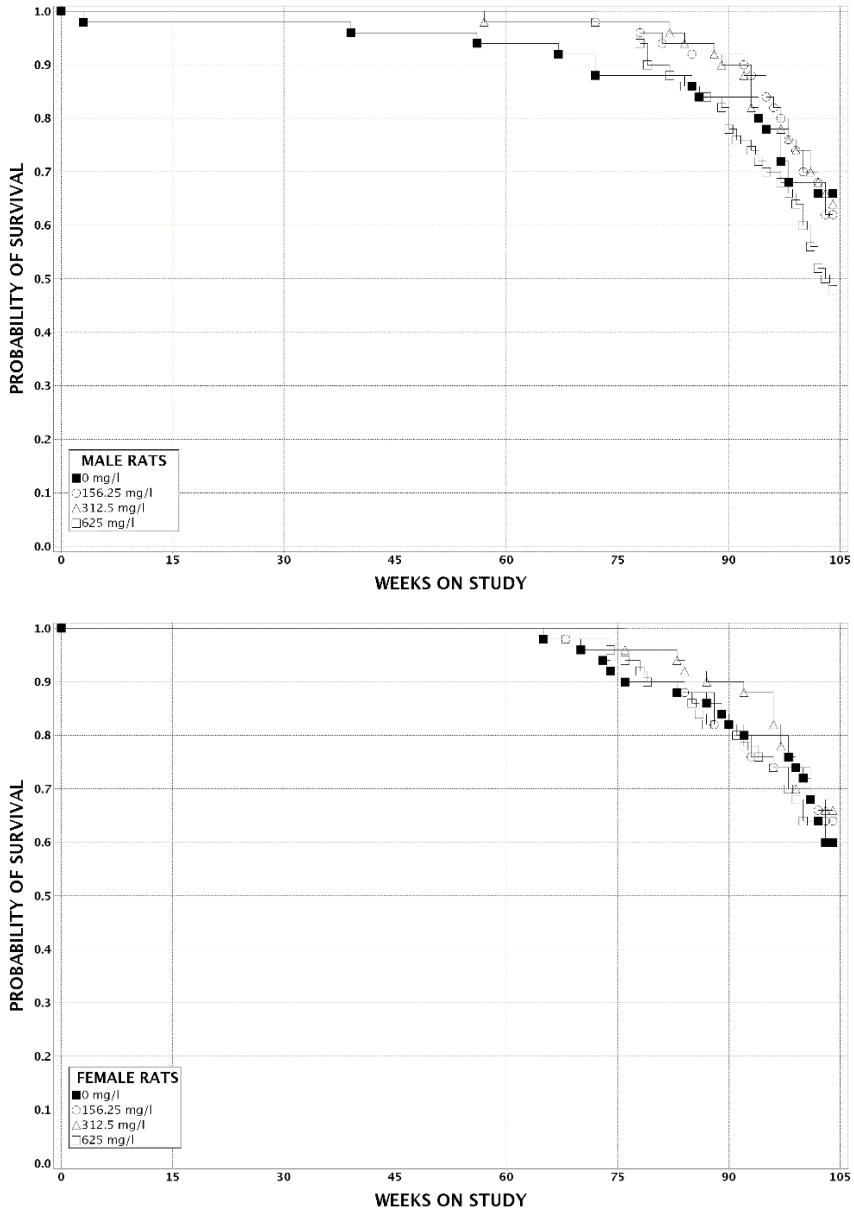

Figure 3. Kaplan-Meier Survival Curves for Rats Exposed to β-Picoline in Drinking Water for Two Years

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of the 156.25 and 312.5 mg/L groups of male and female rats were similar to those of the control groups throughout the study (Table 6 and Table 7; Figure 4). Mean body weights of 625 mg/L males were slightly less than those of controls throughout the study, and were 10% less at the end of the study. In 625 mg/L females, mean body weights were slightly less than those of controls for most of the study, and 9% less for a 16-week period towards the end of the study. Decreased water consumption was evident in 625 mg/L males and females compared to that in the controls throughout the 2-year study (Table 8, Table J-1, and Table J-2). Drinking water concentrations of 156.25, 312.5, and 625 mg/L resulted in average daily doses of approximately 6, 12, and 22 mg β-picoline/kg body weight to male rats and 7, 14, and 26 mg/kg to female rats. No clinical findings related to β-picoline exposure were observed.

Table 6. Mean Body Weights and Survival of Male Rats in the Two-year Drinking Water Study of β-Picoline

Day	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	116	50	115	99	50	116	101	50	115	99	50
8	150	50	147	98	50	148	99	50	142	95	50
15	181	50	179	99	50	181	100	50	171	94	50
22	212	49	208	98	50	208	98	50	195	92	50
29	232	49	229	99	50	230	99	50	215	93	50
36	248	49	248	100	50	248	100	50	234	94	50
43	262	49	263	100	50	263	100	50	247	94	50
50	277	49	276	100	50	278	100	50	259	94	50
57	289	49	289	100	50	290	100	50	271	94	50
64	301	49	301	100	50	302	100	50	281	93	50
71	311	49	311	100	50	313	101	50	289	93	50
78	322	49	321	100	50	323	100	50	298	93	50
85	330	49	329	100	50	330	100	50	305	93	50
113	357	49	354	99	50	357	100	50	332	93	50
141	378	49	376	100	50	383	102	50	352	93	50
169	400	49	399	100	50	402	101	50	374	93	50
197	409	49	409	100	50	416	102	50	387	95	50
225	423	49	420	99	50	429	102	50	400	95	50
253	434	49	435	100	50	441	102	50	410	95	50
281	442	48	441	100	50	453	103	50	422	96	50
309	453	48	453	100	50	462	102	50	431	95	50
337	458	48	460	100	50	467	102	50	436	95	50
365	468	48	467	100	50	477	102	50	445	95	50
393	478	47	478	100	50	488	102	49	452	95	50
421	481	47	480	100	50	487	101	49	453	94	50
449	486	47	487	100	50	493	101	49	457	94	50
477	493	46	490	99	50	499	101	49	463	94	50
505	501	44	499	100	49	506	101	49	471	94	49
533	504	44	506	100	49	510	101	49	472	94	49
561	508	44	508	100	48	511	101	49	475	93	45
589	512	44	517	101	46	514	100	47	474	92	43
617	516	42	516	100	46	516	100	46	474	92	42
645	512	42	509	100	44	504	99	44	470	92	38
673	507	39	512	101	40	508	100	39	465	92	34
701	503	34	497	99	35	510	101	35	453	90	29
Mean for Weeks											
1–13	249	–	247	99	–	248	100	–	232	94	–
14–52	417	–	416	100	–	423	101	–	394	94	–
53–101	498	–	497	100	–	502	101	–	463	93	–

Table 7. Mean Body Weights and Survival of Female Rats in the Two-year Drinking Water Study of β-Picoline

Day	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	99	50	99	100	50	100	101	50	100	101	50
8	117	50	116	99	50	116	99	50	114	97	50
15	132	50	132	100	50	131	99	50	124	94	50
22	144	50	144	101	50	144	100	50	137	95	50
29	152	50	153	101	50	154	101	50	145	95	50
36	162	50	165	102	50	165	101	50	157	97	50
43	170	50	172	101	50	171	101	50	163	96	50
50	175	50	176	101	50	175	100	50	168	96	50
57	180	50	181	101	50	181	101	50	172	96	50
64	184	50	186	101	50	185	100	50	175	95	50
71	188	50	191	101	50	188	100	50	180	96	50
78	192	50	193	101	50	192	100	50	183	95	50
85	195	50	196	101	50	194	100	50	185	95	50
113	207	50	208	101	50	208	100	50	197	95	50
141	218	50	218	100	50	216	99	50	203	93	50
169	223	50	226	101	50	225	101	50	213	96	50
197	233	50	233	100	50	228	98	50	220	95	50
225	238	50	241	101	50	238	100	50	229	96	50
253	247	50	248	100	50	245	99	50	235	95	50
281	253	50	256	101	50	251	99	50	242	96	50
309	264	50	265	100	50	261	99	50	251	95	50
337	270	50	271	100	50	265	98	50	256	95	50
365	282	50	281	100	50	275	98	50	265	94	50
393	294	50	293	100	50	285	97	50	274	93	50
421	302	50	299	99	50	289	96	50	278	92	50
449	311	50	309	100	50	299	96	50	286	92	50
477	319	49	319	100	49	307	96	50	294	92	49
505	328	48	329	100	48	319	97	50	305	93	49
533	340	45	336	99	45	326	96	48	311	91	47
561	344	45	340	99	45	331	96	48	313	91	45
589	348	44	346	100	44	336	97	46	316	91	43
617	352	43	353	100	41	344	98	45	322	91	41
645	353	40	352	100	38	348	99	44	322	91	39
673	350	40	355	102	37	348	100	41	326	93	37
701	346	34	349	101	37	351	101	34	323	93	32
Mean for Weeks											
1–13	161	–	162	101	–	161	100	–	154	96	–
14–52	239	–	241	101	–	237	99	–	227	95	–
53–101	328	–	328	100	–	320	97	–	303	92	–

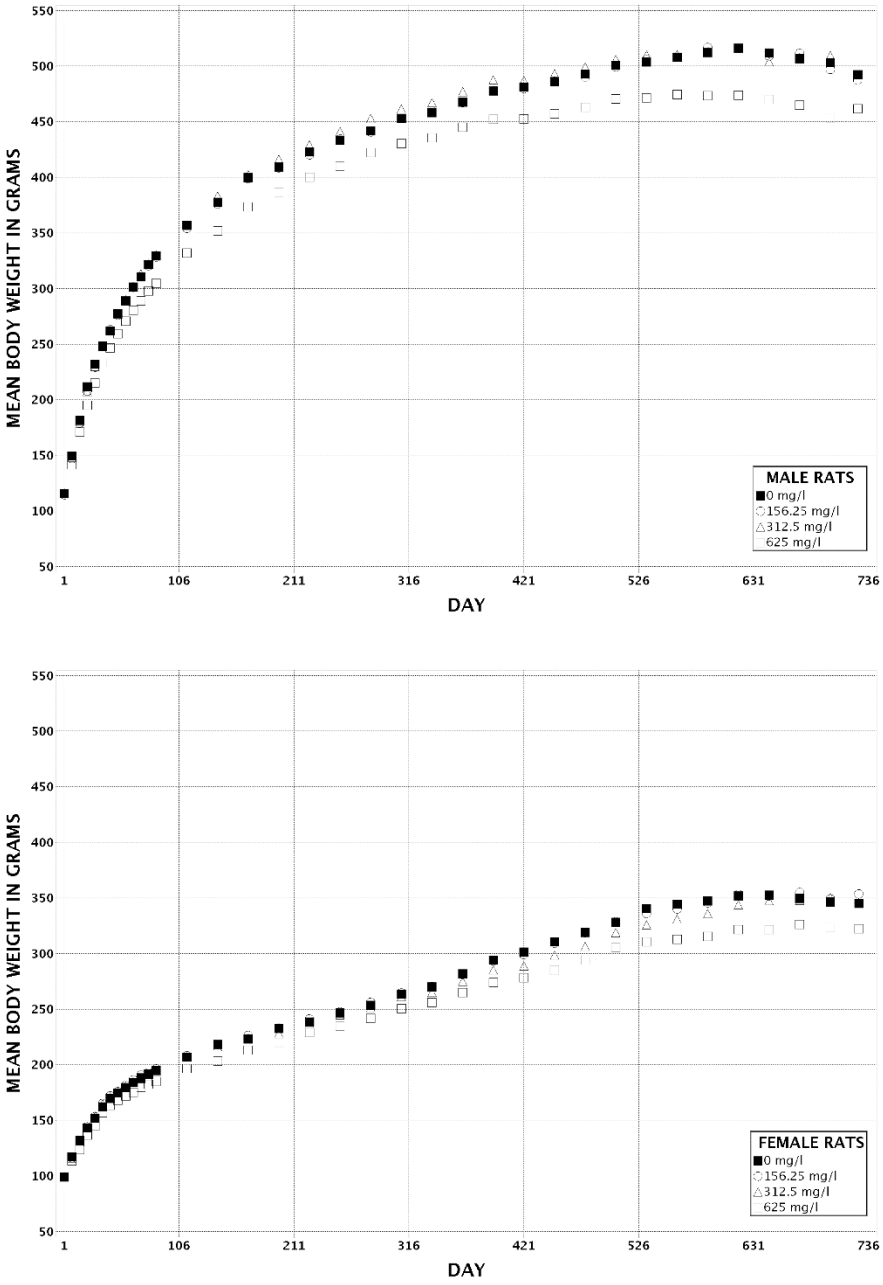

Figure 4. Growth Curves for Rats Exposed to β-Picoline in Drinking Water for Two Years

Table 8. Water and Compound Consumption by Rats in the Two-year Drinking Water Study of β-Picoline

Mean for Weeks	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
Male											
1–13	15.9	249	15.9	247	11	15.4	248	22	13.0	232	38
14–52	15.5	417	15.2	416	6	15.1	423	11	13.1	394	21
53–101	16.9	498	16.3	497	5	15.3	501	10	13.6	463	18
Female											
1–13	11.0	161	11.0	162	11	10.2	161	21	8.9	154	38
14–52	10.6	239	10.3	241	7	9.8	237	13	8.9	227	25
53–101	13.3	328	13.0	328	6	12.1	319	12	11.4	303	23

[a]Grams of water consumed per animal per day.
[b]Milligrams of β-picoline consumed per kilogram body weight per day.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the lung and oral cavity. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Lung: The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in exposed groups of males were similar to that in the controls (Table 9, Table A-1, and Table A-2). In males, alveolar/bronchiolar carcinoma occurred in the 312.5 and 625 mg/L groups; four incidences occurred in 312.5 mg/L males and two incidences occurred in 625 mg/L males. The increased incidence of alveolar/bronchiolar carcinoma in 312.5 mg/L males was not significant compared to the controls (0/50), but this neoplasm has not occurred in the historical controls for drinking water studies and the 8% incidence slightly exceeded the historical control range for all routes of administration (Table 9 and Table A-3). In females, alveolar/bronchiolar adenoma occurred in all exposed groups, but not in the controls (Table 9, Table B-1, and Table B-2). A single incidence of alveolar/bronchiolar carcinoma occurred in a 156.25 mg/L female. Incidences of alveolar/bronchiolar adenoma in the 625 mg/L females were significantly greater than those in the controls and slightly exceeded the historical control ranges for drinking water studies and all routes of administration (Table 9 and Table B-3). The incidences of alveolar epithelium hyperplasia and squamous metaplasia were increased, but not significantly, in all exposed groups of females compared to those in the controls (Table 9 and Table B-4).

Table 9. Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Rats in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Male				
Number Examined Microscopically	50	50	50	50
Alveolar/bronchiolar Adenoma[a,b]	3	5	1	2
Alveolar/bronchiolar Carcinoma (includes multiple)[c]				
Overall rate[d]	0/50 (0%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	8.8%	4.7%
Terminal rate[f]	0/33 (0%)	0/31 (0%)	3/32 (9%)	1/24 (4%)
First incidence (days)	–[h]	–	615	607
Poly-3 test[g]	P = 0.095	–[i]	P = 0.069	P = 0.238
Alveolar/bronchiolar Adenoma or Carcinoma[j]				
Overall rate	3/50 (6%)	5/50 (10%)	5/50 (10%)	4/50 (8%)
Adjusted rate	7.0%	11.0%	11.0%	9.3%
Terminal rate	2/33 (6%)	5/31 (16%)	4/32 (13%)	2/24 (8%)
First incidence (days)	600	726 (T)	615	607
Poly-3 test	P = 0.478	P = 0.387	P = 0.389	P = 0.501
Female				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia	7 (1.3)[k]	14 (1.5)	14 (2.1)	11 (1.2)
Alveolar Epithelium, Metaplasia, Squamous	0	3 (1.3)	1 (2.0)	1 (1.0)
Alveolar/bronchiolar Adenoma[l]				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	6.7%	4.4%	11.5%
Terminal rate	0/30 (0%)	1/32 (3%)	2/33 (6%)	4/30 (13%)
First incidence (days)	–	526	727 (T)	638
Poly-3 test	P = 0.029	P = 0.122	P = 0.245	P = 0.030
Alveolar/bronchiolar Carcinoma[m]	0	1	0	0
Alveolar/bronchiolar Adenoma or Carcinoma[n]				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	9.0%	4.4%	11.5%
Terminal rate	0/30 (0%)	2/32 (6%)	2/33 (6%)	4/30 (13%)
First incidence (days)	–	526	727 (T)	638
Poly-3 test	P = 0.050	P = 0.063	P = 0.245	P = 0.030

(T) Terminal kill.
[a]Number of animals with lesion.
[b]Historical incidence for 2-year drinking water studies with untreated control groups (mean ± standard deviation): 7/100 (7.0% ± 1.4%), range 6%–8%; all routes: 31/1,249 (2.5% ± 2.6%), range 0%–8%.

[c]Historical incidence for drinking water studies: 0/100; all routes: 15/1,249 (1.2% ± 1.4%), range 0%–6%.
[d]Number of animals with neoplasm per number of animals with lung examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill.
[h]Not applicable; no neoplasms in animal group.
[i]Value of statistic cannot be computed.
[j]Historical incidence for drinking water studies: 7/100 (7.0% ± 1.4%), range 6%–8%; all routes: 45/1,249 (3.6% ± 2.8%), range 0%–10%.
[k]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[l]Historical incidence for drinking water studies: 4/100 (4.0% ± 5.7%), range 0%–8%; all routes: 25/1,200 (2.1% ± 2.9%), range 0%–8%.
[m]Historical incidence for drinking water studies: 0/100; all routes: 3/1,200 (0.3% ± 0.7%), range 0%–2%.
[n]Historical incidence for drinking water studies: 4/100 (4.0% ± 5.7%), range 0%–8%; all routes: 27/1,200 (2.3% ± 2.9%), range 0%–8%.

Alveolar bronchiolar adenomas were discrete, moderately cellular masses that distorted and replaced alveolar architecture and obliterated alveolar spaces. The proliferative cells generally formed irregular papillary structures supported by delicate, fibrovascular stroma. The cells were polygonal, moderately uniform in size, and had small to moderate amounts of eosinophilic cytoplasm. Nuclei were round to oval, moderately uniform, and mitoses were few. Carcinomas were generally not well circumscribed and contained stratification of the neoplastic epithelium, solid areas of growth, cellular pleomorphism and atypia, or anaplastic cells associated with a scirrhous reaction. Alveolar epithelium hyperplasia consisted of focal areas of increased numbers of Type II pneumocytes lining alveolar walls in the absence of cellular atypia with maintenance of alveolar architecture. Alveolar epithelium metaplasia consisted of a change from the normal cubiodal epithelium lining the alveoli to a flattened, squamous epithelium.

Oral Cavity: An expanded review of the oral cavity for all proliferative lesions was performed because squamous cell neoplasms were observed grossly during necropsy. One squamous cell carcinoma of the tongue each occurred in 312.5 and 625 mg/L males (0 mg/L, 0/50; 156.25 mg/L, 0/50; 312.5 mg/L, 1/50; 625 mg/L, 1/50); none occurred in females (Table A-1 and Table B-1). Incidences of squamous cell papilloma or carcinoma (combined) in the oral mucosa or tongue (combined) were increased in 312.5 and 625 mg/L males and females (males: 1/50, 1/50, 2/50, 2/50; females: 0/50, 0/50, 1/50, 2/50). Due to the low incidences, these lesions were not considered to be related to treatment.

Mice

Three-month Study

All mice survived to the end of the study (Table 10). Final mean body weights and body weight gains of exposed groups of male and female mice were generally similar to those of the control groups (Table 10 and Figure 5). Water consumption by the exposed and control groups was generally similar; water consumption by 1,250 mg/L males was initially less than that by the controls due to palatability. Drinking water concentrations of 78, 156, 312, 625, and 1,250 mg/L resulted in average daily doses of approximately 10, 20, 37, 77, and 148 mg β-picoline/kg body weight to males and 9, 18, 38, 72, and 134 mg/kg to females. There were no clinical findings related to β-picoline exposure.

Table 10. Survival, Body Weights, and Water Consumption of Mice in the Three-month Drinking Water Study of β-Picoline[a]

Concentration (mg/L)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)	Water Consumption Week 1	Water Consumption Week 13
Male							
0	10/10	21.8 ± 0.4	38.5 ± 1.3	16.7 ± 1.0		3.7	3.3
78	10/10	22.1 ± 0.7	39.5 ± 0.8	17.4 ± 1.1	103	3.6	3.2
156	10/10	22.3 ± 0.5	39.9 ± 1.2	17.6 ± 0.9	104	3.7	3.7
312	10/10	21.8 ± 0.5	39.9 ± 1.3	18.1 ±1.1	104	3.6	3.3
625	10/10	21.8 ± 0.5	39.6 ± 1.1	17.8 ± 1.0	103	3.6	3.3
1,250	10/10	22.2 ± 0.5	37.9 ± 1.0	15.7 ± 0.8	98	3.4	3.1
Female							
0	10/10	18.3 ± 0.6	27.2 ± 0.9	8.8 ± 0.9		2.1	2.6
78	10/10	17.9 ± 0.4	27.6 ± 1.0	9.7 ± 0.7	102	2.3	2.8
156	10/10	18.6 ± 0.5	30.1 ± 1.0	11.5 ± 0.8	111	2.3	2.6
312	10/10	18.2 ± 0.5	28.0 ± 1.1	9.7 ± 0.8	103	2.3	2.8
625	10/10	18.1 ± 0.5	27.6 ± 0.5	9.5 ± 0.6	102	2.3	2.7
1,250	10/10	18.2 ± 0.6	27.9 ± 0.9	9.7 ± 0.7	103	2.1	2.6

[a]Weights and weight changes are given as mean ± standard error. Water consumption is expressed as grams per animal per day. Differences in weights and weight changes from the control group are not significant by Dunnett's test.
[b]Number of animals surviving at 14 weeks/number initially in group.

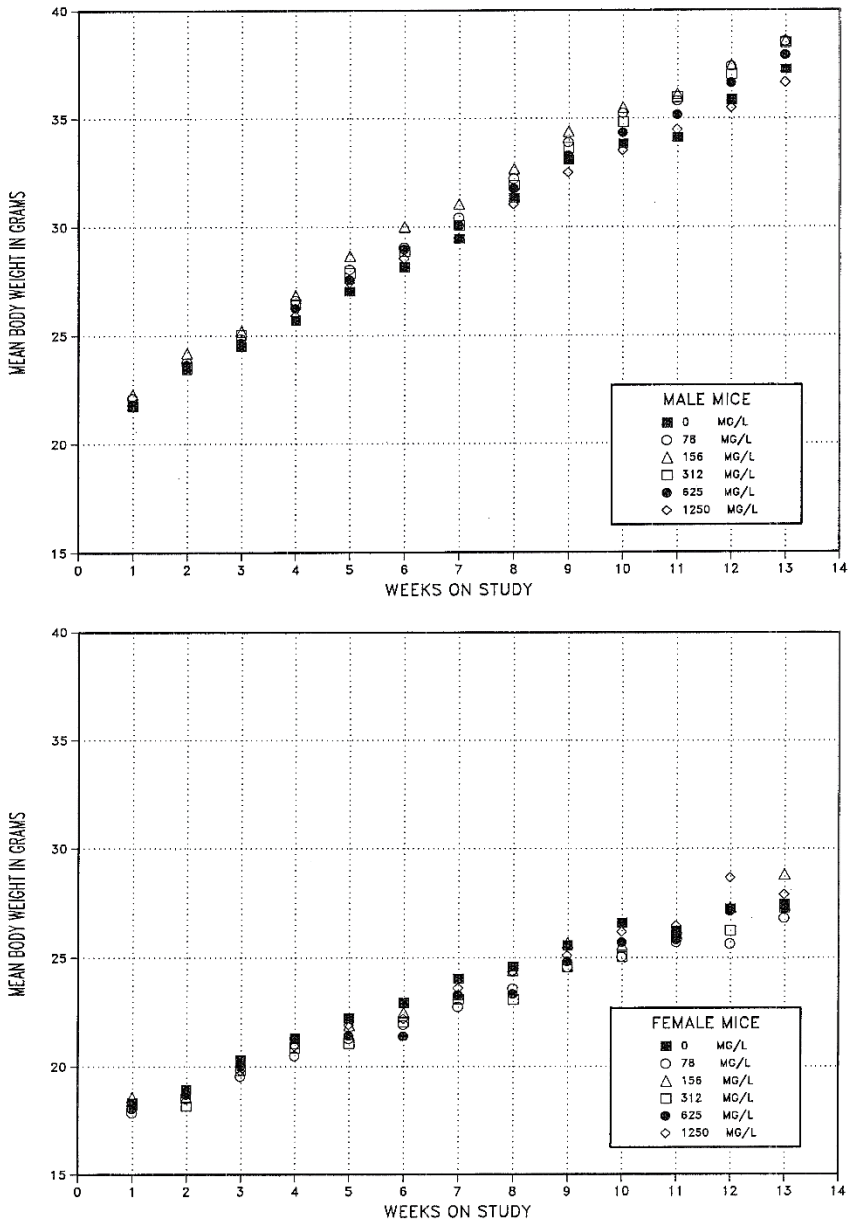

Figure 5. Growth Curves for Mice Exposed to β-Picoline in Drinking Water for Three Months

There were no changes in the hematology data that were considered attributable to β-picoline administration (Table F-2).

Absolute and relative lung weights of 1,250 mg/L females were significantly less than those of the controls (Table G-2).

There were no significant differences in sperm parameters of male mice or in the estrous cycles of female mice exposed to 312, 625, or 1,250 mg/L when compared to the controls (Table H-4 and Table H-5).

No gross or microscopic lesions were observed that were considered to be due to β-picoline exposure.

Exposure Concentration Selection Rationale: Based on the absence of exposure-related effects in the 3-month study in mice, β-picoline exposure concentrations selected for the 2-year drinking water study in mice were 312.5, 625, and 1,250 mg/L.

Two-year Study

Survival

Estimates of the 2-year survival probabilities for male and female mice are shown in Table 11 and in the Kaplan-Meier survival curves (Figure 6). Survival of all exposed groups was similar to that of the control groups; however, there was a positive trend in the survival of male mice.

Table 11. Survival of Mice in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Male				
Animals initially in study	50	50	50	50
Moribund	6	4	8	7
Natural deaths	20	20	15	10
Animals surviving to study termination	24	26	27	33
Percent probability of survival at end of study[a]	48	52	54	66
Mean survival (days)[b]	645	674	671	688
Survival analysis[c]	P = 0.041N	P = 0.503N	P = 0.456N	P = 0.058N
Female				
Animals initially in study	50	50	50	50
Moribund	4	3	1	4
Natural deaths	8	15	14	13
Animals surviving to study termination	38	32	35[d]	33
Percent probability of survival at end of study	76	64	70	66
Mean survival (days)	702	694	700	694
Survival analysis	P = 0.446	P = 0.294	P = 0.656	P = 0.325

[a]Kaplan-Meier determinations.
[b]Mean of all deaths (uncensored, censored, and terminal kill).
[c]The result of the life table trend test[27] is in the control column, and the results of the life table pairwise comparisons[26] with the controls are in the exposed group columns. A negative trend or lower mortality in an exposure group is indicated by **N**.
[d]Includes one animal that died during the last week of the study.

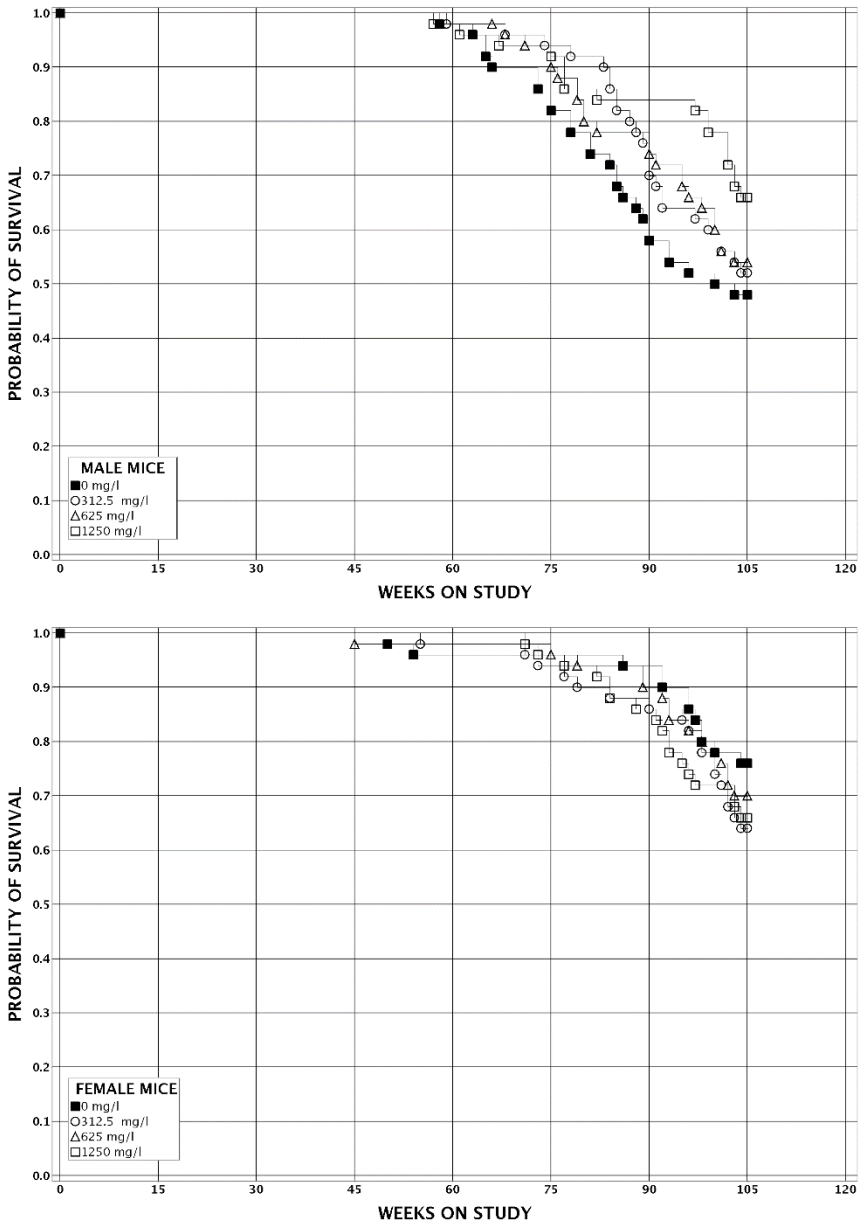

Figure 6. Kaplan-Meier Survival Curves for Mice Exposed to β-Picoline in Drinking Water for Two Years

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 1,250 mg/L males were at least 10% less than those of the control group after week 57, and those of 1,250 mg/L females were generally 10% less after week 13 (Table 12 and Table 13 and Figure 7). Water consumption was lower in 625 and 1,250 mg/L males and 1,250 mg/L females compared to that in the controls after the first 13 weeks of the study (Table 14, Table J-3, and Table J-4). Drinking water concentrations of 312.5, 625, and 1,250 mg/L resulted in average daily doses of approximately 26, 50, and 92 mg β-picoline/kg body weight to males and 18, 37, and 68 mg/kg to females. No chemical-related clinical findings were observed.

Table 12. Mean Body Weights and Survival of Male Mice in the Two-year Drinking Water Study of β-Picoline

Day	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	23.7	50	23.8	100	50	23.6	99	50	23.7	100	50
8	25.8	50	25.5	99	50	25.2	98	50	25.2	98	50
15	27.2	50	27.0	99	50	26.5	98	50	26.6	98	50
22	28.8	50	28.7	100	50	28.1	98	50	28.1	98	50
29	30.9	50	30.5	99	50	29.8	96	50	29.9	97	50
36	33.0	50	32.5	98	50	31.6	96	50	31.5	95	50
43	35.0	50	34.5	99	50	33.3	95	50	33.3	95	50
50	36.6	50	35.9	98	50	34.8	95	50	34.7	95	50
57	38.1	50	37.5	99	50	36.2	95	50	36.2	95	50
64	39.7	50	39.0	98	50	37.7	95	50	37.7	95	50
71	41.2	50	40.7	99	50	39.3	95	50	39.3	95	50
78	42.9	50	42.2	98	50	40.8	95	50	40.8	95	50
85	44.1	50	43.2	98	50	41.8	95	50	41.8	95	50
113	48.0	50	46.8	98	50	45.4	95	50	45.3	94	50
141	49.5	50	48.5	98	50	47.2	95	50	46.5	94	50
169	50.2	50	49.1	98	50	47.9	95	50	47.1	94	50
197	51.9	50	50.8	98	50	49.6	96	50	48.5	94	50
225	52.9	50	52.0	98	50	50.5	95	50	49.1	93	50
253	54.0	50	52.9	98	50	51.3	95	50	49.7	92	50
281	55.1	50	54.0	98	50	52.2	95	50	50.3	91	50
309	55.9	50	55.3	99	50	53.2	95	50	51.0	91	50
337	56.7	50	55.7	98	50	53.2	94	50	51.5	91	50
365	56.7	50	55.9	99	50	54.1	95	50	52.1	92	50
393	57.1	50	56.5	99	50	53.9	94	50	51.9	91	50
421	57.0	49	56.0	98	49	53.4	94	50	51.1	90	49
449	56.3	48	55.6	99	49	52.9	94	50	50.8	90	48
477	56.3	45	55.6	99	48	53.1	94	48	49.9	89	47
505	56.3	44	55.0	98	48	52.9	94	47	48.8	87	47
533	55.4	41	54.1	98	47	52.5	95	44	47.2	85	46
561	54.3	39	52.5	97	46	51.4	95	40	46.1	85	43
589	53.2	36	51.4	97	43	50.3	95	39	44.9	84	42
617	50.3	32	51.5	102	39	49.0	98	39	42.7	85	42
645	48.5	29	51.1	105	32	46.5	96	36	40.6	84	42
673	46.8	26	48.6	104	32	45.0	96	33	37.9	81	42
701	43.0	25	47.7	111	30	42.7	99	30	36.8	86	39
Mean for Weeks											
1–13	34.4	–	33.9	99	–	33.0	96	–	33.0	96	–
14–52	52.7	–	51.7	98	–	50.1	95	–	48.8	93	–
53–101	53.2	–	53.2	100	–	50.6	95	–	46.2	87	–

Table 13. Mean Body Weights and Survival of Female Mice in the Two-year Drinking Water Study of β-Picoline

Day	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of Controls)	No. of Survivors
1	18.6	50	18.8	101	50	18.7	101	50	18.5	100	50
8	19.7	50	19.7	100	50	19.2	98	50	19.1	97	50
15	20.7	50	20.6	99	50	20.2	97	50	20.0	97	50
22	21.4	50	21.7	101	50	21.3	100	50	20.8	97	50
29	22.3	50	22.7	102	50	22.1	99	50	21.5	96	50
36	23.4	50	23.4	100	50	22.7	97	50	22.4	96	50
43	24.5	50	24.6	101	50	23.7	97	50	23.3	95	50
50	25.1	50	25.0	100	50	24.4	98	50	23.6	94	50
57	26.6	50	26.6	100	50	25.5	96	50	24.8	93	50
64	27.5	50	27.7	101	50	27.1	99	50	25.8	94	50
71	29.3	50	29.3	100	50	28.5	97	50	26.5	90	50
78	30.5	50	30.6	100	50	30.1	99	50	27.9	92	50
85	31.8	50	32.1	101	50	31.1	98	50	28.9	91	50
113	37.6	50	37.5	100	50	36.5	97	50	33.7	90	50
141	43.3	50	42.9	99	50	41.2	95	50	38.8	90	50
169	47.3	50	47.2	100	50	45.0	95	50	42.3	89	50
197	50.4	50	50.2	100	50	47.8	95	50	45.3	90	50
225	53.3	50	53.3	100	50	51.0	96	50	48.2	90	50
253	55.2	50	55.6	101	50	52.9	96	50	50.3	91	50
281	57.5	50	57.3	100	50	54.8	95	50	51.9	90	50
309	58.6	50	58.4	100	50	56.0	96	49	53.0	90	50
337	59.7	50	59.5	100	50	57.1	96	49	54.7	92	50
365	61.5	49	60.5	98	50	58.5	95	49	55.5	90	50
393	62.8	48	62.6	100	49	60.2	96	49	56.8	90	50
421	63.8	48	63.6	100	49	61.3	96	49	57.5	90	50
449	65.0	48	64.8	100	49	62.2	96	49	58.1	89	50
477	65.6	48	65.4	100	49	62.5	95	49	59.0	90	50
505	67.3	48	66.1	98	48	63.4	94	49	59.3	88	49
533	67.4	48	65.5	97	46	63.1	94	48	60.6	90	47
561	66.2	48	65.1	98	45	64.1	97	47	60.0	91	47
589	65.3	48	65.2	100	44	64.0	98	47	60.3	92	44
617	64.9	47	64.8	100	44	63.7	98	46	58.6	90	43
645	63.9	45	63.6	100	43	62.5	98	44	56.5	89	41
673	61.2	43	61.0	100	41	60.2	98	41	56.5	92	37
701	60.6	39	57.4	95	37	57.5	95	40	53.3	88	36
Mean for Weeks											
1–13	24.7	–	24.8	100	–	24.2	98	–	23.3	95	–
14–52	51.4	–	51.3	100	–	49.1	96	–	46.5	90	–
53–101	64.3	–	63.5	99	–	61.8	96	–	57.8	90	–

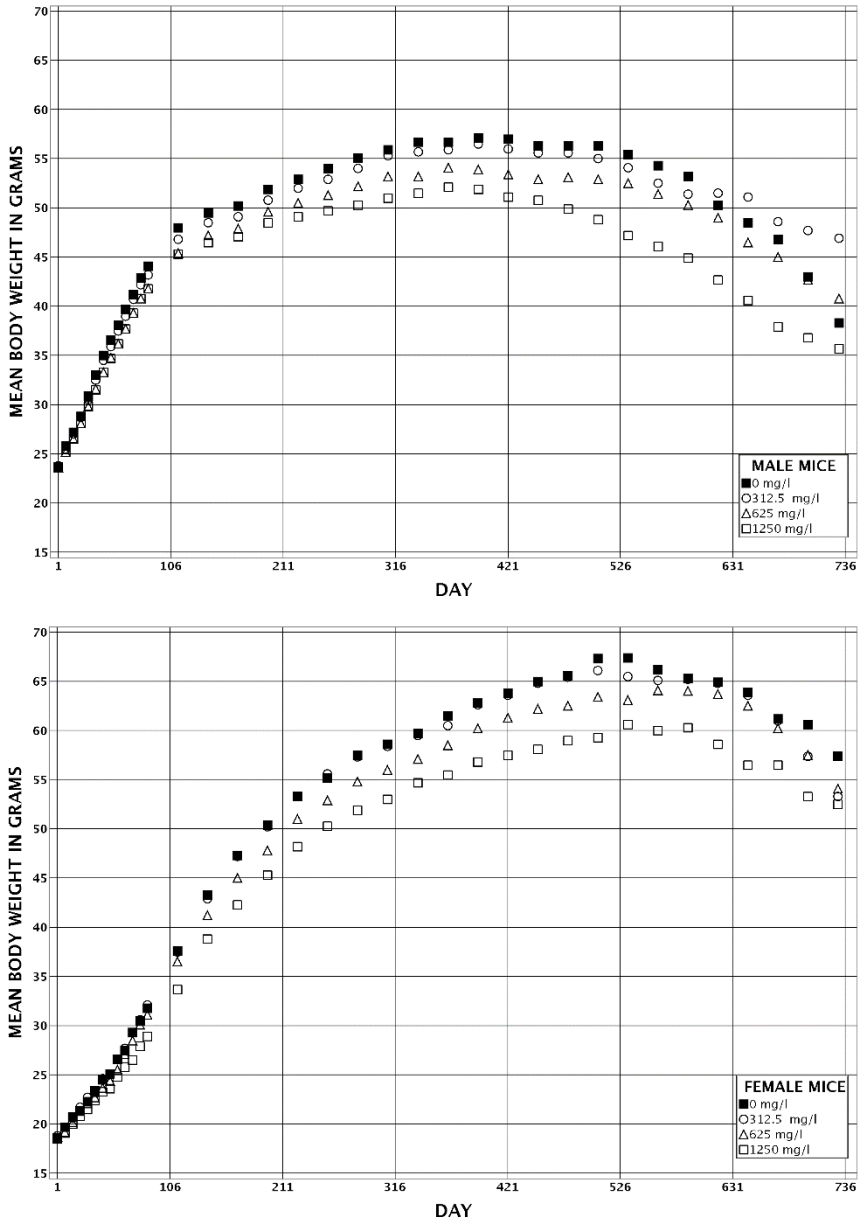

Figure 7. Growth Curves for Mice Exposed to β-Picoline in Drinking Water for Two Years

Table 14. Water and Compound Consumption by Mice in the Two-year Drinking Water Study of β-Picoline

Mean for Weeks	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
Male											
1–13	3.7	34.4	3.6	33.9	34	3.5	33.0	69	3.4	33.0	132
14–52	4.1	52.7	3.7	51.7	23	3.5	50.1	43	3.2	48.8	81
53–101	4.7	53.2	4.5	53.2	26	4.0	50.6	50	3.3	46.2	90
Female											
1–13	2.8	24.7	2.6	24.8	34	2.5	24.2	66	2.3	23.3	123
14–52	2.5	51.4	2.5	51.3	15	2.4	49.1	31	2.2	46.5	60
53–101	3.3	64.3	3.4	63.4	17	3.3	61.8	34	2.8	57.8	60

[a]Grams of water consumed per animal per day.
[b]Milligrams of β-picoline consumed per kilogram body weight per day.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the liver, lung, and nose. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: Compared to controls, the incidences of hepatocellular adenoma were significantly increased in 312.5 and 625 mg/L females, with a significant increase in multiple hepatocellular adenomas in 312.5 mg/L females (Table 15, Table D-1, and Table D-2). The incidences of hepatocellular carcinoma were significantly increased in all exposed groups of females compared to that in the controls. The incidences of hepatocellular carcinoma in all exposed groups of females exceeded the historical control range for drinking water studies and the incidence in 625 mg/L females exceeded the historical control range for all routes of administration. The incidences of hepatoblastoma in all exposed groups of females were greater than that in the controls; although not significantly increased, these incidences exceeded the historical control ranges for drinking water studies and for all routes of administration. A single incidence of multiple hepatoblastoma occurred in each of the 625 and 1,250 mg/L groups of females. The combined incidences of hepatocellular carcinoma and hepatoblastoma were significantly increased in all exposed groups of females and exceeded the historical control ranges for drinking water studies and for all routes of administration in the 625 and 1,250 mg/L groups. There were no treatment-related effects on the incidences of liver neoplasms in male mice.

Table 15. Incidences of Neoplasms of the Liver in Female Mice in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Number Examined Microscopically	49	50	50	50
Hepatocellular Adenoma, Multiple[a]	27	43**	36	30
Hepatocellular Adenoma (includes multiple)[b]				
Overall rate[c]	38/49 (78%)	46/50 (92%)	46/50 (92%)	39/50 (78%)
Adjusted rate[d]	80.4%	94.9%	93.1%	81.5%
Terminal rate[e]	31/38 (82%)	31/32 (97%)	35/35 (100%)	26/33 (79%)
First incidence (days)	598	494	309	509
Poly-3 test[f]	P = 0.403N	P = 0.025	P = 0.052	P = 0.551
Hepatocellular Carcinoma, Multiple	5	7	10	5
Hepatocellular Carcinoma (includes multiple)[g]				
Overall rate	11/49 (22%)	20/50 (40%)	26/50 (52%)	23/50 (46%)
Adjusted rate	23.6%	43.9%	55.3%	50.9%
Terminal rate	7/38 (18%)	14/32 (44%)	21/35 (60%)	18/33 (55%)
First incidence (days)	639	533	549	586
Poly-3 test	P = 0.006	P = 0.031	P < 0.001	P = 0.005
Hepatoblastoma, Multiple	0	0	1	1
Hepatoblastoma (includes multiple)[h]				
Overall rate	1/49 (2%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	2.2%	6.7%	8.8%	9.1%
Terminal rate	0/38 (0%)	1/32 (3%)	4/35 (11%)	3/33 (9%)
First incidence (days)	674	662	729 (T)	721
Poly-3 test	P = 0.140	P = 0.295	P = 0.174	P = 0.166
Hepatocellular Carcinoma or Hepatoblastoma[i]				
Overall rate	12/49 (24%)	21/50 (42%)	28/50 (56%)	24/50 (48%)
Adjusted rate	25.7%	45.8%	59.5%	53.1%
Terminal rate	7/38 (18%)	14/32 (44%)	23/35 (66%)	19/33 (58%)
First incidence (days)	639	533	549	586
Poly-3 test	P = 0.005	P = 0.033	P < 0.001	P = 0.005

**Significantly different (P ≤ 0.01) from the control group by the Poly-3 test.
(T) Terminal kill.
[a]Number of animals with lesion.
[b]Historical incidence for 2-year drinking water studies with untreated control groups (mean ± standard deviation: 52/98 (53.1% ± 34.6%), range 29%–78%; all routes: 380/1,195 (31.8% ± 21.4%), range 2%–78%.
[c]Number of animals with neoplasm per number of animals with liver examined microscopically.
[d]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[e]Observed incidence at terminal kill.
[f]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend in an exposure group is indicated by **N**.
[g]Historical incidence for drinking water studies: 19/98 (19.4% ± 4.3%), range 16%–22%; all routes: 144/1,195 (12.1% ± 10.8%), range 0%–46%.
[h]Historical incidence for drinking water studies: 1/98 (1.0% ± 1.4%), range 0%–2%; all routes: 4/1,195 (0.3% ± 0.8%), range 0%–2%.
[i]Historical incidence for drinking water studies: 20/98 (20.4% ± 5.8%), range 16%–24%; all routes: 148/1,195 (12.4% ± 11.2%), range 0%–46%.

The hepatocellular adenomas were typically well-demarcated nodular masses that caused compression of the surrounding parenchyma on all sides and lacked normal architecture. They were composed of variably pleomorphic hepatocytes arranged in irregular cords that intersected at sharp angles with the cords of the surrounding parenchyma. The majority of the hepatocellular carcinomas were composed of pleomorphic hepatocytes arranged in a trabecular pattern, with trabeculae that were three or more cells thick. Many contained large blood-filled spaces and areas of necrosis. Solid and glandular patterns were also noted. Hepatoblastomas were often located within or adjacent to hepatocellular carcinomas or adenomas. In such cases, only a diagnosis of hepatoblastoma was recorded. Hepatoblastoma was diagnosed when all or a portion of a mass was composed of a densely cellular proliferation of small cells containing round to oval hyperchromatic nuclei and scant cytoplasm, often arranged in nests and palisading around blood-filled vascular spaces.

Lung: The incidence of alveolar/bronchiolar adenoma was significantly increased in 625 mg/L males compared to that in the controls (Table 16, Table C-1, and Table C-2) and the time to first incidence in this exposed group (470 days) was 260 days shorter than in the controls. In females, there was a positive trend in the incidences of alveolar/bronchiolar adenoma with an incidence in the 1,250 mg/L group that exceeded the historical control ranges for drinking water studies and for all routes of administration (Table 16, Table D-1, and Table D-2). The incidences of alveolar/bronchiolar carcinoma in all exposed groups of females were greater than that in the controls, but these increases were not statistically significant. Multiple alveolar/bronchiolar carcinomas occurred in two 312.5 mg/L, two 625 mg/L, and four 1,250 mg/L females. There was a positive trend in the combined incidences of alveolar/bronchiolar adenoma or carcinoma in females, and the incidence in the 1,250 mg/L group was significantly greater than that in the controls. The combined incidences of alveolar/bronchiolar adenoma or carcinoma were increased without significance in all exposed groups of males, exceeded the historical control ranges for drinking water studies and for all routes combined in 625 mg/L males, and exceeded the historical control range for drinking water studies, but not for all routes combined, in 312.5 mg/L males. The incidence of alveolar epithelium hyperplasia was significantly increased in 1,250 mg/L females and bronchiole hyperplasia was observed in a few 625 and 1,250 mg/L females.

Table 16. Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Male				
Number Examined Microscopically	50	50	50	50
Alveolar Epithelium, Hyperplasia[a]	4 (1.8)[b]	6 (1.2)	6 (2.7)	7 (1.7)
Alveolar/bronchiolar Adenoma, Multiple	0	0	2	2
Alveolar/bronchiolar Adenoma (includes multiple)[c]				
Overall rate[d]	6/50 (12%)	11/50 (22%)	16/50 (32%)	8/50 (16%)
Adjusted rate[e]	16.3%	25.8%	36.1%	18.2%
Terminal rate[f]	6/24 (25%)	5/26 (19%)	8/27 (30%)	6/33 (18%)
First incidence (days)	730 (T)	627	470	709
Poly-3 test[g]	P = 0.504N	P = 0.222	P = 0.037	P = 0.526
Alveolar/bronchiolar Carcinoma, Multiple	0	2	0	3
Alveolar/bronchiolar Carcinoma (includes multiple)[h]				
Overall rate	9/50 (18%)	9/50 (18%)	8/50 (16%)	9/50 (18%)
Adjusted rate	23.2%	21.2%	19.4%	20.6%
Terminal rate	5/24 (21%)	5/26 (19%)	6/27 (22%)	8/33 (24%)
First incidence (days)	455	512	628	724
Poly-3 test	P = 0.448N	P = 0.516N	P = 0.443N	P = 0.490N
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	14/50 (28%)	19/50 (38%)	21/50 (42%)	15/50 (30%)
Adjusted rate	36.2%	42.8%	47.0%	34.2%
Terminal rate	10/24 (42%)	9/26 (35%)	12/27 (44%)	12/33 (36%)
First incidence (days)	455	512	470	709
Poly-3 test	P = 0.382N	P = 0.343	P = 0.213	P = 0.517N
Female				
Number Examined Microscopically	50	50	49	50
Alveolar Epithelium, Hyperplasia	2 (1.5)	4 (2.5)	3 (1.3)	8* (1.8)
Bronchiole, Hyperplasia	0	0	3 (2.0)	1 (2.0)
Alveolar/bronchiolar Adenoma, Multiple	0	1	0	1
Alveolar/bronchiolar Adenoma (includes multiple)[j]				
Overall rate	5/50 (10%)	6/50 (12%)	4/49 (8%)	11/50 (22%)
Adjusted rate	10.9%	13.5%	8.9%	24.5%
Terminal rate	5/38 (13%)	5/32 (16%)	3/35 (9%)	8/33 (24%)
First incidence (days)	729 (T)	700	669	533
Poly-3 test	P = 0.046	P = 0.477	P = 0.511N	P = 0.075
Alveolar/bronchiolar Carcinoma, Multiple	0	2	2	4
Alveolar/bronchiolar Carcinoma (includes multiple)[k]				

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Overall rate	7/50 (14%)	8/50 (16%)	10/49 (20%)	13/50 (26%)
Adjusted rate	15.2%	17.9%	21.6%	28.5%
Terminal rate	6/38 (16%)	6/32 (19%)	7/35 (20%)	8/33 (24%)
First incidence (days)	669	700	522	509
Poly-3 test	P = 0.061	P = 0.471	P = 0.297	P = 0.096
Alveolar/bronchiolar Adenoma or Carcinoma[l]				
Overall rate	11/50 (22%)	13/50 (26%)	13/49 (27%)	21/50 (42%)
Adjusted rate	23.8%	29.1%	28.1%	45.4%
Terminal rate	10/38 (26%)	11/32 (34%)	10/35 (29%)	14/33 (42%)
First incidence (days)	669	700	522	509
Poly-3 test	P = 0.015	P = 0.368	P = 0.407	P = 0.022

*Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test.
(T) Terminal kill.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.
[c]Historical incidence for 2-year drinking water studies with untreated control groups (mean ± standard deviation): 21/100 (21.0% ± 12.7%), range 12%–30%; all routes: 172/1,150 (15.0% ± 6.9%), range 2%–30%.
[d]Number of animals with neoplasm per number of animals with lung examined microscopically.
[e]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[f]Observed incidence at terminal kill.
[g]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[h]Historical incidence for drinking water studies: 12/100 (12.0% ± 8.5%), range 6%–18%; all routes: 144/1,150 (12.5% ± 7.1%),range 4%–24%.
[i]Historical incidence for drinking water studies: 30/100 (30.0% ± 2.8%), range 28%–32%; all routes: 301/1,150 (26.2% ± 6.3%), range 14%–40%.
[j]Historical incidence for drinking water studies: 6/100 (6.0% ± 5.7%), range 2%–10%; all routes: 60/1,196 (5.0% ± 3.6%), range 0%–12%.
[k]Historical incidence for drinking water studies: 9/100 (9.0% ± 7.1%), range 4%–14%; all routes: 44/1,196 (3.7% ± 3.3%), range 0%–14%.
[l]Historical incidence for drinking water studies: 13/100 (13.0% ± 12.7%), range 4%–22%; all routes: 100/1,196 (8.4% ± 4.3%), range 2%–22%.

Alveolar/bronchiolar adenomas consisted of a well-demarcated, densely cellular proliferation of cuboidal to columnar cells supported by a fine fibrovascular stroma and forming short projections into alveolar spaces and causing compression of the surrounding parenchyma. Alveolar/bronchiolar carcinomas tended to be larger and were characterized by irregular, often poorly demarcated proliferations of pleomorphic cuboidal to columnar cells containing pleomorphic nuclei with occasional mitoses. The cells formed irregular papillary structures and/or solid clusters, with most neoplasms containing a combination of the two patterns. Alveolar epithelium and bronchiole hyperplasia were typically focal lesions characterized by increased numbers of large, plump cuboidal cells (type II pneumocytes) containing large, hyperchromatic nuclei, with maintenance of the alveolar and bronchiole architectures.

Nose: The incidences of olfactory epithelium atrophy in 1,250 mg/L females and olfactory epithelium respiratory metaplasia in 625 mg/L males and in 1,250 mg/L males and females were significantly increased compared to the controls (Table 17, Table C-4, and Table D-5).

Table 17. Incidences of Nonneoplastic Lesions of the Nose in Mice in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Male				
Number Examined Microscopically	50	50	50	50
Olfactory Epithelium, Atrophy[a]	3 (1.3)[b]	4 (1.3)	8 (1.5)	7 (1.6)
Olfactory Epithelium, Metaplasia, Respiratory	8 (1.0)	12 (1.1)	30** (1.3)	41** (1.7)
Female				
Number Examined Microscopically	49	44	49	47
Olfactory Epithelium, Atrophy	1 (1.0)	2 (1.0)	2 (1.0)	7* (1.3)
Olfactory Epithelium, Metaplasia, Respiratory	2 (1.0)	2 (1.0)	7 (1.0)	14** (1.2)

*Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test.
**$P \leq 0.01$.
[a]Number of animals with lesion.
[b]Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked.

Nose olfactory epithelium atrophy was characterized by thinning of the pseudostratified columnar epithelium and respiratory metaplasia was characterized by replacement of olfactory epithelium with a single layer of ciliated columnar (respiratory) epithelium. Respiratory epithelium hyperplasia consisted of hyperplastic epithelium that was thickened due to increased cellularity with a folded or scalloped appearance due to mucosal invaginations with the formation of intraepithelial crypt-like structures or "pseudoglands." This hyperplastic epithelium quite often had a rugose appearance of the mucosal surface due to numerous deep invaginations with a notable expansion of the mucosa and lamina propria. Pseudoglands were sometimes filled with inflammatory cells and mucus.

Genetic Toxicology

β-Picoline was tested in three independent bacterial gene mutation studies, and negative results were obtained in all studies (Table E-1 and Table E-2). In the first study (with concentrations ranging from 85.4 to 8,540 μg/plate), no increases in the numbers of mutant colonies were seen in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without 10% S9 derived from induced hamster or rat liver. In the second study, negative results were obtained over a concentration range of 100 to 10,000 μg/plate in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 with and without 10% or 30% S9 derived from induced hamster or rat liver. In the third study, which tested the same chemical lot 11108CI that was used in the 3-month and 2-year studies, negative results were obtained over a concentration range of 1,000 to 10,000 μg/plate in *S. typhimurium* strains TA98 and TA100 and 100 to 5,000 μg/plate in *Escherichia coli* WP2 *uvrA*/pKM101, with and without 10% rat liver S9.

In vivo, no significant increases in the frequencies of micronucleated normochromatic erythrocytes, an indicator of chromosomal damage, were observed in peripheral blood of male or female B6C3F1/N mice exposed to 78 to 1,250 mg β-picoline/L in drinking water for 3 months (Table E-3). No significant alterations in the percentage of circulating polychromatic erythrocytes (reticulocytes) were observed, suggesting that β-picoline did not induce bone marrow toxicity over the exposure concentration range tested.

Discussion

β-Picoline was nominated by the National Institute of Environmental Health Sciences for toxicologic and carcinogenicity evaluation based on its high production volume and potential for human exposure. β-Picoline is used as a solvent, reagent, and intermediate in various commercial and industrial processes, and has been identified as a contaminant in drinking water and cigarette smoke. β-Picoline is structurally similar to pyridine, differing only in the presence of a methyl group on the 3-carbon in β-picoline. In previous studies by the National Toxicology Program[17], exposure to pyridine induced renal tubule neoplasms in male F344/N rats, and malignant hepatocellular neoplasms in male and female B6C3F1 mice. Minimal toxicology data are available, and no carcinogenicity studies are reported in the literature for β-picoline.

In the 3-month rat study, the estimated daily doses of β-picoline in rats exposed to drinking water concentrations of 78, 156, 312, 625, or 1,250 mg/L were 6, 11, 22, 38, or 70 mg β-picoline/kg body weight in males and 6, 12, 23, 38, or 64 mg/kg in females. Decreased body weights and palatability-related decreases in water consumption were observed in both sexes at 625 and 1,250 mg/L. In males, there were minor and transient increases in hematocrit values, erythrocyte counts, and hemoglobin, urea nitrogen, albumin, and total protein concentrations on day 4 that were consistent with the observed palatability-related decreases in water consumption. These effects were not observed in females.

In the kidney of male rats, there were increased concentrations of renal α2u-globulin at 312 mg/L or greater and increased severities of chronic progressive nephropathy at 625 mg/L or greater and hyaline droplet accumulation in the proximal tubules at 1,250 mg/L. These findings in the kidney were suggestive of weak α2u-globulin mediated nephropathy in male rats and are consistent with α2u-globulin-related kidney effects observed in the 3-month pyridine drinking water study[17]. In the pyridine study, there were significantly increased incidences of protein casts, chronic inflammation, and mineralization and increased severities of renal tubule regeneration in male F344/N rats exposed to 500 or 1,000 ppm pyridine (equivalent to 55 and 90 mg/kg) and renal tubule hyaline degeneration and granular casts at 1,000 ppm. In contrast to pyridine, which significantly induced renal tubule adenomas and carcinomas in males at 400 ppm (equivalent to 33 mg/kg), no renal neoplasms were observed in the 2-year β-picoline study. While both β-picoline and pyridine similarly induced α2u-globulin-related lesions at 3-months, the mechanism for the differences in chronic renal responses between β-picoline and pyridine is unclear. Previous evaluation of results from 3-month and 2-year NTP studies with α2u-globulin-inducing chemicals suggests that none of the characteristic nonneoplastic endpoints or increases in α2u-globulin concentration observed in the 3-month studies were predictive of tumor outcome in the corresponding 2-year studies[60].

While pyridine induces hepatic hypertrophy, chronic inflammation, centrilobular degeneration, and pigmentation in male and female F344/N and male Wistar rats[17], there were no treatment-related liver lesions observed following exposure to β-picoline for 3 months. There was, however, a dose-dependent increase in the activity of 7-pentoxyresorufin-O-dealkylase activity, a marker for cytochrome P4502B1, with significant increases observed at 312 mg/L or greater in males and 156 mg/L or greater in females. While the induction of liver toxicity differed between β-picoline and pyridine, the modulation of CYP2B1 by β-picoline was consistent with dose- and time-dependent expression demonstrated for pyridine[57-59].

In the 3-month mouse study, exposure to β-picoline in drinking water at concentrations of 78, 156, 312, 625, or 1,250 mg/L resulted in estimated daily doses of 10, 20, 37, 77, or 148 mg/kg in males and 9, 18, 38, 72, or 134 mg/kg in females. Mice were less sensitive to the effects of β-picoline on body weight and drinking water palatability than rats. At estimated average daily doses nearly two-fold greater than in rats, there were no treatment-related effects on survival, body weights, water consumption, or incidences of gross or histopathologic lesions in the 3-month mouse study. The absence of kidney lesions in male and female mice and female rats further suggests a role for α2u-globulin in the lesions observed in the male rats.

The significantly higher probability of extended estrus of female rats in the 312 and 625 mg/L groups as observed through Markov transition matrix analyses of estrous cyclicity suggests a potential for β-picoline to be a reproductive toxicant in female rats exposed to these concentrations.

Based on the lower final mean body weights (16% and 14% less than controls in 1,250 mg/L males and females, respectively), decreased water consumption in both sexes, and the increased severity of nephropathy observed in males at 1,250 mg/L, β-picoline concentrations of 156.25, 312.5, and 625 mg/L in drinking water were selected for the 2-year rat study. These exposures in the 2-year study resulted in average daily doses of 6, 12, and 22 mg/kg β-picoline to male rats and 7, 14, and 26 mg/kg to female rats. Survival rates were comparable between the control and exposed groups of rats. Mean body weights were slightly lower in 625 mg/L males throughout the course of the study, and were decreased by a maximum of 10% at the end of the study. Mean body weights in 625 mg/L females were 9% less than those in controls for a 16-week period toward the end of the study.

In the 2-year rat study, the lung was a target organ. In the female rats, alveolar/bronchiolar adenomas were observed in all of the exposed groups, while none were observed in the concurrent controls. There was a significant increase in the incidence of this neoplasm in the 625 mg/L females that exceeded the historical control ranges for drinking water studies and for all routes of administration. One alveolar/bronchiolar carcinoma occurred in a 156.25 mg/L female. The incidences of alveolar epithelium hyperplasia and metaplasia were increased in exposed groups of females but were not significantly greater than those in the controls. Alveolar/bronchiolar carcinoma occurred in four 312.5 mg/L males and two 625 mg/L males but did not occur in controls or in males exposed to 156.25 mg/L. While the combined incidences of alveolar/bronchiolar adenoma or carcinoma in males were similar between the control and exposed groups, these observations may suggest a treatment-related progression from benign tumors to malignancy. These data suggest that increased incidences of alveolar/bronchiolar adenoma are specific to females, and that females are more sensitive to the induction of lung neoplasms by β-picoline.

Based on the lack of treatment-related effects observed in the 3-month study in mice, β-picoline concentrations of 312.5, 625, and 1,250 mg/L in drinking water were selected for the 2-year study in mice. These exposure concentrations in the 2-year study resulted in average daily doses of 26, 50, and 92 mg/kg β-picoline to male mice and 18, 37, and 68 mg/kg to female mice. These calculated doses reflect a 26% to 30% greater dose of β-picoline to males than to females. Survival of exposed groups of females was similar to that of the controls and there was an exposure concentration-dependent trend for slightly increased survival in males. In groups exposed to 1,250 mg/L, mean body weights were generally 10% less than those in controls with

maximal decreases of 19% in males and 12% in females. Additionally, water consumption by 625 and 1,250 mg/L males and 1,250 mg/L females was less than that by the controls after the first 13 weeks of the study.

In the 2-year mouse study, liver and lung were target organs for β-picoline in females. In female mice, the incidences of hepatocellular carcinoma were significantly increased in all exposed groups. A single hepatoblastoma, a neoplasm with a somewhat rare background incidence in female B6C3F1/N mice, was observed in a control female. Hepatoblastomas occurred in three 312.5 mg/L females and four occurred in each of the 625 and 1,250 mg/L groups of females; all of these incidences exceeded the historical control ranges for drinking water studies and for all routes of administration. One female in the 625 mg/L group and one female in the 1,250 mg/L group had multiple hepatoblastomas. There were also increased numbers of animals with multiple hepatocellular adenomas. There was a high background incidence of hepatocellular adenoma (78%) in the current 2-year study, which complicates the determination of a treatment-related effect. However, the evidence for the hepatocarcinogenicity of β-picoline is primarily supported by increased incidences of hepatocellular carcinoma and hepatoblastoma. The increased incidences of those neoplasms suggest a treatment-mediated effect on the progression from benign to malignant tumors. Additionally, metastatic hepatocellular carcinomas were observed with a greater frequency in the lungs of all groups of exposed females (0 mg/L, 1/50; 312.5 mg/L, 5/50; 625 mg/L, 8/49; 1,250 mg/L, 4/50; Table D-1). A metastatic hepatoblastoma was also in one 1,250 mg/L female. There were no treatment-related effects on incidences of liver neoplasms in male mice. There were also no significant increases in the incidences of any nonneoplastic lesions in the liver of males or females.

The induction of liver neoplasms by β-picoline in female B6C3F1/N mice was similar to the effects observed in the 2-year study of pyridine[17]. In that study, daily pyridine exposure in the drinking water induced hepatocellular neoplasms, including hepatoblastomas, in female mice at doses (15, 35, and 70 mg/kg) similar to those in the current study of β-picoline (18, 37, and 68 mg/kg). Additionally, pyridine administration significantly increased the incidences of multiple hepatocellular adenoma and carcinoma. Similar to β-picoline, there were no significant effects of treatment with pyridine on the incidences of nonneoplastic lesions in the liver. In contrast to β-picoline, the hepatic carcinogenicity of pyridine was not specific to females. Exposure to pyridine for 2 years resulted in significantly increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in male mice, including treatment-related increases in multiplicities.

Similar to the effects observed in females in the 2-year rat study, the lung was a target organ in females in the 2-year mouse study. There was a positive trend in the incidences of alveolar/bronchiolar adenoma in female mice and a dose-dependent association between exposure and the incidences of alveolar/bronchiolar carcinoma. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 1,250 mg/L females was significantly increased compared to that in the controls. Additionally, incidences of multiple alveolar/bronchiolar adenoma and multiple alveolar/bronchiolar carcinoma occurred in most of the exposed groups of females, but no multiple lung neoplasms occurred in the controls. The incidence of alveolar epithelium hyperplasia, a potential precursor lesion in the neoplastic development of an adenoma, was significantly increased in 1,250 mg/L females. In male mice, the incidence of alveolar/bronchiolar adenoma was significantly increased in the 625 mg/L group compared to controls, and the time to first incidence was 260 days shorter than in controls.

Multiple alveolar/bronchiolar adenomas were also observed in two males each from the 625 and 1,250 mg/L groups. Multiple alveolar/bronchiolar carcinomas were observed in two 312.5 mg/L males and three 1,250 mg/L males. However, the incidences in the 312.5 and 1,250 mg/L groups were not significantly different than that in the concurrent controls, and were within the historical control ranges for drinking water studies and for all routes of administration.

The lung was a sex-specific target organ for β-picoline in rats and mice; benign and malignant alveolar/bronchiolar neoplasms occurred in females. While the incidences of alveolar/bronchiolar adenoma in all exposed groups of male mice were greater than those in the controls, there was no dose-response relationship, and most of the incidences were within the historical control ranges. However, the increased incidence of alveolar/bronchiolar adenoma in 625 mg/L male mice was statistically significant, slightly exceeded the historical control ranges for drinking water studies and for all routes of administration, and occurred markedly earlier than in the controls. As a result, it was considered that the increased incidences of alveolar/bronchiolar adenoma in male mice may have been related to treatment. While lung lesions in the current study reflected differences between β-picoline and pyridine, liver lesions observed in female mice in the current study were consistent with the effects observed in both sexes of mice for pyridine[17].

Administration of β-picoline in drinking water induced significantly increased incidences of olfactory epithelium atrophy in the nose of 1,250 mg/L female mice and olfactory epithelium respiratory metaplasia in 625 and 1,250 mg/L males and 1,250 mg/L females. Since β-picoline is considered a volatile organic compound, it is possible that inhalation of a volatilized fraction may have contributed to the observed olfactory lesions. Alternatively, the effects in the nasal cavity may have resulted from systemic exposure. These nasal effects were only observed in mice; the reason for the species specificity of these effects is unclear.

Conclusions

Under the conditions of these 2-year drinking water studies, there was *no evidence of carcinogenic activity*[a] of β-picoline in male F344/N rats exposed to 156.25, 312.5, or 625 mg/L. There was *some evidence of carcinogenic activity* of β-picoline in female F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of β-picoline in male B6C3F1/N mice based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was *clear evidence of carcinogenic activity* of β-picoline in female B6C3F1/N mice based on the increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in the lung and of hepatocellular carcinoma and hepatoblastoma in the liver.

Exposure to β-picoline caused increased incidences of nonneoplastic lesions of the lung in female mice and the nose in male and female mice.

[a]See Explanation of Levels of Evidence of Carcinogenic Activity. A summary of the Peer Review Panel comments and the public discussion on this Technical Report appears in Appendix M.

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Appendix A. Summary of Lesions in Male Rats in the Two-year Drinking Water Study of β-Picoline

Tables

Table A-1. Summary of the Incidence of Neoplasms in Male Rats in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	12	15	23
Natural deaths	8	7	3	3
Survivors				
Terminal kill	33	31	32	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(45)	(47)	(48)	(48)
Intestine large, colon	(47)	(49)	(49)	(49)
Adenoma	1 (2%)	–	–	–
Intestine large, rectum	(47)	(49)	(49)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Intestine small, duodenum	(48)	(49)	(49)	(50)
Intestine small, ileum	(46)	(47)	(48)	(49)
Intestine small, jejunum	(45)	(47)	(48)	(49)
Leiomyoma	–	–	–	1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Hepatocellular adenoma	2 (4%)	1 (2%)	–	2 (4%)
Hepatocellular adenoma, multiple	1 (2%)	–	–	–
Hepatocellular carcinoma	–	–	1 (2%)	–
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Mesentery	(4)	(8)	(4)	(6)
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (17%)
Osteosarcoma, metastatic, uncertain primary site	–	1 (13%)	–	–
Pancreas	(49)	(50)	(50)	(48)
Adenoma, mixed cell	1 (2%)	1 (2%)	–	–
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Acinus, adenoma	–	–	–	1 (2%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma	–	–	–	1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	–	1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(2)	(1)
Squamous cell carcinoma	–	–	1 (50%)	1 (100%)
Squamous cell papilloma	–	–	1 (50%)	–
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Carcinoma, metastatic, salivary glands	–	–	–	1 (2%)
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	–	1 (2%)	1 (2%)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Adrenal medulla	(50)	(49)	(49)	(50)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Pheochromocytoma benign	4 (8%)	5 (10%)	6 (12%)	5 (10%)
Pheochromocytoma complex	1 (2%)	–	–	–
Pheochromocytoma malignant	–	–	2 (4%)	–
Bilateral, pheochromocytoma benign	1 (2%)	–	–	–
Bilateral, pheochromocytoma malignant	–	–	1 (2%)	–
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	1 (2%)	3 (6%)	2 (4%)
Parathyroid gland	(50)	(47)	(48)	(48)
Pituitary gland	(50)	(49)	(50)	(49)
Pars distalis, adenoma	28 (56%)	35 (71%)	28 (56%)	36 (73%)
Pars intermedia, adenoma	–	–	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	12 (24%)	10 (20%)	4 (8%)	8 (16%)
C-cell, carcinoma	1 (2%)	3 (6%)	1 (2%)	–
Follicular cell, adenoma	–	–	1 (2%)	–
Follicular cell, carcinoma	1 (2%)	1 (2%)	–	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
General Body System				
Peritoneum	(2)	(1)	(1)	(2)
Osteosarcoma, metastatic, uncertain primary site	–	1 (100%)	–	–
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	–	1 (2%)	2 (4%)	–
Carcinoma	2 (4%)	1 (2%)	2 (4%)	–
Prostate	(49)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	22 (44%)	20 (40%)	26 (52%)	24 (48%)
Interstitial cell, adenoma	13 (26%)	18 (36%)	16 (32%)	18 (36%)
Tunic, leiomyosarcoma	–	–	1 (2%)	–
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(9)	(11)	(11)	(8)
Deep cervical, carcinoma, metastatic, harderian gland	1 (11%)	–	–	–
Iliac, chordoma, metastatic, uncertain primary site	–	–	–	1 (13%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Carcinoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Thymus	(49)	(49)	(45)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Adenoma	–	–	1 (2%)	–
Carcinoma	–	1 (2%)	–	–
Fibroadenoma	1 (2%)	1 (2%)	2 (4%)	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	2 (4%)	2 (4%)	–
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Keratoacanthoma	5 (10%)	5 (10%)	7 (14%)	5 (10%)
Squamous cell carcinoma	–	1 (2%)	–	–
Squamous cell papilloma	–	–	1 (2%)	–
Subcutaneous tissue, fibroma	3 (6%)	4 (8%)	2 (4%)	5 (10%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	–	–	–
Subcutaneous tissue, hemangioma	–	1 (2%)	–	–
Subcutaneous tissue, lipoma	–	–	2 (4%)	–
Subcutaneous tissue, sarcoma	–	–	1 (2%)	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Sarcoma	–	–	1 (2%)	–
Skeletal muscle	(0)	(0)	(0)	(1)
Fibrous histiocytoma	–	–	–	1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	5 (10%)	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma	–	–	3 (6%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	–	–	1 (2%)	–
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Carcinoma, metastatic, preputial gland	1 (2%)	–	–	–
Carcinoma, metastatic, thyroid gland	–	1 (2%)	1 (2%)	–
Carcinoma, metastatic, Zymbal’s gland	–	–	1 (2%)	–
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Pheochromocytoma malignant, metastatic, adrenal medulla	–	–	1 (2%)	–
Rhabdomyosarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Respiratory epithelium, adenoma	–	1 (2%)	–	–
Trachea	(49)	(50)	(50)	(50)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Special Senses System				
Ear	(2)	(0)	(0)	(0)
Neural crest tumor	1 (50%)	–	–	–
Eye	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Harderian gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	–	–	–
Zymbal's gland	(1)	(0)	(1)	(1)
Adenoma	–	–	–	1 (100%)
Carcinoma	1 (100%)	–	1 (100%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Hemangiosarcoma	–	1 (2%)	–	–
Capsule, osteosarcoma, metastatic, uncertain primary site	–	1 (2%)	–	–
Renal tubule, adenoma	–	1 (2%)	–	1 (2%)
Renal tubule, adenoma, multiple	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skeletal muscle	–	–	–	1 (2%)
Leiomyoma	–	–	1 (2%)	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	14 (28%)	19 (38%)	16 (32%)	14 (28%)
Lymphoma malignant	–	–	1 (2%)	–
Mesothelioma malignant	4 (8%)	2 (4%)	1 (2%)	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	50	50	50
Total primary neoplasms	130	142	144	132
Total animals with benign neoplasms	45	48	49	50
Total benign neoplasms	103	113	110	113
Total animals with malignant neoplasms	22	25	30	19
Total malignant neoplasms	26	29	34	19
Total animals with metastatic neoplasms	3	2	5	4
Total metastatic neoplasms	9	12	5	15
Total animals with malignant neoplasms of uncertain primary site	1	1	2	1

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Total animals with neoplasms uncertain- benign or malignant	1	–	–	–
Total uncertain neoplasms	1	–	–	–

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table A-2. Statistical Analysis of Primary Neoplasms in Male Rats in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	5/50 (10%)	5/49 (10%)	6/49 (12%)	5/50 (10%)
Adjusted rate[b]	11.8%	11.3%	13.5%	11.6%
Terminal rate[c]	5/33 (15%)	5/31 (16%)	5/31 (16%)	3/24 (13%)
First incidence (days)	726 (T)	726 (T)	657	586
Poly-3 test[d]	P = 0.545	P = 0.603N	P = 0.530	P = 0.625N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	0/50 (0%)	0/49 (0%)	3/49 (6%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	6.8%	0.0%
Terminal rate	0/33 (0%)	0/31 (0%)	1/31 (3%)	0/24 (0%)
First incidence (days)	–[e]	–	646	–
Poly-3 test	P = 0.520	–[f]	P = 0.127	–
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	6/50 (12%)	5/49 (10%)	9/49 (18%)	5/50 (10%)
Adjusted rate	14.0%	11.3%	20.1%	11.6%
Terminal rate	5/33 (15%)	5/31 (16%)	6/31 (19%)	3/24 (13%)
First incidence (days)	600	726 (T)	646	586
Poly-3 test	P = 0.525N	P = 0.476N	P = 0.315	P = 0.500N
Liver: Hepatocellular Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	2/50 (4%)
Adjusted rate	7.1%	2.2%	0.0%	4.7%
Terminal rate	3/33 (9%)	0/31 (0%)	0/32 (0%)	1/24 (4%)
First incidence (days)	726 (T)	717	–	720
Poly-3 test	P = 0.450N	P = 0.282N	P = 0.108N	P = 0.502N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	7.1%	2.2%	2.2%	4.7%
Terminal rate	3/33 (9%)	0/31 (0%)	1/32 (3%)	1/24 (4%)
First incidence (days)	726 (T)	717	726 (T)	720
Poly-3 test	P = 0.483N	P = 0.282N	P = 0.284N	P = 0.502N

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	5/50 (10%)	1/50 (2%)	2/50 (4%)
Adjusted rate	7.0%	11.0%	2.2%	4.7%
Terminal rate	2/33 (6%)	5/31 (16%)	1/32 (3%)	1/24 (4%)
First incidence (days)	600	726 (T)	726 (T)	628
Poly-3 test	P = 0.247N	P = 0.387	P = 0.288N	P = 0.503N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	8.8%	4.7%
Terminal rate	0/33 (0%)	0/31 (0%)	3/32 (9%)	1/24 (4%)
First incidence (days)	–	–	615	607
Poly-3 test	P = 0.095	–	P = 0.069	P = 0.238
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	5/50 (10%)	4/50 (8%)
Adjusted rate	7.0%	11.0%	11.0%	9.3%
Terminal rate	2/33 (6%)	5/31 (16%)	4/32 (13%)	2/24 (8%)
First incidence (days)	600	726 (T)	615	607
Poly-3 test	P = 0.478	P = 0.387	P = 0.389	P = 0.501
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.4%	2.2%	6.7%	0.0%
Terminal rate	1/33 (3%)	0/31 (0%)	3/32 (9%)	0/24 (0%)
First incidence (days)	726 (T)	684	726 (T)	–
Poly-3 test	P = 0.422N	P = 0.745N	P = 0.327	P = 0.501N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.4%	4.4%	6.7%	0.0%
Terminal rate	1/33 (3%)	1/31 (3%)	3/32 (9%)	0/24 (0%)
First incidence (days)	726 (T)	684	726 (T)	–
Poly-3 test	P = 0.345N	P = 0.524	P = 0.327	P = 0.501N
Pancreatic Islets: Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	9.4%	2.2%	6.6%	4.7%
Terminal rate	4/33 (12%)	0/31 (0%)	2/32 (6%)	1/24 (4%)
First incidence (days)	726 (T)	567	615	691
Poly-3 test	P = 0.388N	P = 0.156N	P = 0.463N	P = 0.337N

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	28/50 (56%)	35/49 (71%)	28/50 (56%)	36/49 (73%)
Adjusted rate	61.4%	73.7%	59.6%	79.5%
Terminal rate	19/33 (58%)	25/31 (81%)	19/32 (59%)	18/23 (78%)
First incidence (days)	499	498	615	499
Poly-3 test	P = 0.078	P = 0.144	P = 0.513N	P = 0.041
Preputial Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	4.6%	4.4%	8.8%	0.0%
Terminal rate	0/33 (0%)	2/31 (7%)	3/32 (9%)	0/24 (0%)
First incidence (days)	600	726 (T)	692	–
Poly-3 test	P = 0.246N	P = 0.676N	P = 0.360	P = 0.241N
Skin: Keratoacanthoma				
Overall rate	5/50 (10%)	5/50 (10%)	7/50 (14%)	5/50 (10%)
Adjusted rate	11.7%	10.9%	15.4%	11.7%
Terminal rate	4/33 (12%)	4/31 (13%)	4/32 (13%)	3/24 (13%)
First incidence (days)	683	544	646	664
Poly-3 test	P = 0.512	P = 0.583N	P = 0.424	P = 0.631
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	5/50 (10%)	5/50 (10%)	7/50 (14%)	5/50 (10%)
Adjusted rate	11.7%	10.9%	15.4%	11.7%
Terminal rate	4/33 (12%)	4/31 (13%)	4/32 (13%)	3/24 (13%)
First incidence (days)	683	544	646	664
Poly-3 test	P = 0.512	P = 0.583N	P = 0.424	P = 0.631
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	7/50 (14%)	5/50 (10%)
Adjusted rate	11.7%	13.1%	15.4%	11.7%
Terminal rate	4/33 (12%)	5/31 (16%)	4/32 (13%)	3/24 (13%)
First incidence (days)	683	544	646	664
Poly-3 test	P = 0.559	P = 0.552	P = 0.424	P = 0.631
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	6/50 (12%)	8/50 (16%)	9/50 (18%)	5/50 (10%)
Adjusted rate	13.9%	17.4%	19.8%	11.7%
Terminal rate	4/33 (12%)	6/31 (19%)	6/32 (19%)	3/24 (13%)
First incidence (days)	600	544	646	664
Poly-3 test	P = 0.414N	P = 0.439	P = 0.325	P = 0.508N

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	3/50 (6%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	6.9%	8.8%	4.4%	11.7%
Terminal rate	2/33 (6%)	3/31 (10%)	0/32 (0%)	4/24 (17%)
First incidence (days)	391	684	646	618
Poly-3 test	P = 0.309	P = 0.527	P = 0.478N	P = 0.347
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	4/50 (8%)	4/50 (8%)	3/50 (6%)	5/50 (10%)
Adjusted rate	9.2%	8.8%	6.6%	11.7%
Terminal rate	3/33 (9%)	3/31 (10%)	1/32 (3%)	4/24 (17%)
First incidence (days)	391	684	646	618
Poly-3 test	P = 0.415	P = 0.616N	P = 0.474N	P = 0.490
Testes: Adenoma				
Overall rate	35/50 (70%)	38/50 (76%)	42/50 (84%)	42/50 (84%)
Adjusted rate	77.7%	79.5%	87.5%	87.5%
Terminal rate	27/33 (82%)	25/31 (81%)	31/32 (97%)	23/24 (96%)
First incidence (days)	504	589	569	543
Poly-3 test	P = 0.088	P = 0.519	P = 0.152	P = 0.150
Thyroid Gland (C-Cell): Adenoma				
Overall rate	12/50 (24%)	10/50 (20%)	4/50 (8%)	8/50 (16%)
Adjusted rate	28%	21.7%	8.8%	18.7%
Terminal rate	10/33 (30%)	7/31 (23%)	3/32 (9%)	6/24 (25%)
First incidence (days)	676	638	681	568
Poly-3 test	P = 0.141N	P = 0.328N	P = 0.018N	P = 0.220N
Thyroid Gland: (C-Cell): Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.4%	6.5%	2.2%	0.0%
Terminal rate	0/33 (0%)	2/31 (7%)	1/32 (3%)	0/24 (0%)
First incidence (days)	713	567	726 (T)	–
Poly-3 test	P = 0.197N	P = 0.333	P = 0.748N	P = 0.501N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	13/50 (26%)	12/50 (24%)	5/50 (10%)	8/50 (16%)
Adjusted rate	30.3%	25.7%	11.0%	18.7%
Terminal rate	10/33 (30%)	8/31 (26%)	4/32 (13%)	6/24 (25%)
First incidence (days)	676	567	681	568
Poly-3 test	P = 0.081N	P = 0.402N	P = 0.022N	P = 0.156N

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
All Organs: Mononuclear Leukemia				
Overall rate	14/50 (28%)	19/50 (38%)	16/50 (32%)	14/50 (28%)
Adjusted rate	31.4%	39.3%	33.7%	30.5%
Terminal rate	9/33 (27%)	8/31 (26%)	8/32 (25%)	4/24 (17%)
First incidence (days)	467	544	569	499
Poly-3 test	P = 0.388N	P = 0.280	P = 0.492	P = 0.554N
All Organs: Malignant Mesothelioma				
Overall rate	4/50 (8%)	2/50 (4%)	1/50 (2%)	0/50 (0%)
Adjusted rate	9.0%	4.4%	2.2%	0.0%
Terminal rate	0/33 (0%)	1/31 (3%)	1/32 (3%)	0/24 (0%)
First incidence (days)	273	693	726 (T)	–
Poly-3 test	P = 0.031N	P = 0.325N	P = 0.173N	P = 0.066N
All Organs: Benign Tumors				
Overall rate	45/50 (90%)	48/50 (96%)	49/50 (98%)	50/50 (100%)
Adjusted rate	95.5%	96.1%	99.7%	100.0%
Terminal rate	32/33 (97%)	30/31 (97%)	32/32 (100%)	24/24 (100%)
First incidence (days)	391	498	569	499
Poly-3 test	P = 0.066	P = 0.648	P = 0.218	P = 0.179
All Organs: Malignant Tumors				
Overall rate	23/50 (46%)	26/50 (52%)	30/50 (60%)	19/50 (38%)
Adjusted rate	49.3%	53.5%	60.9%	40.1%
Terminal rate	13/33 (39%)	13/31 (42%)	17/32 (53%)	5/24 (21%)
First incidence (days)	273	544	393	499
Poly-3 test	P = 0.187N	P = 0.420	P = 0.173	P = 0.243N
All Organs: Benign or Malignant Tumors				
Overall rate	48/50 (96%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	98.0%	100.0%	100.0%	100.0%
Terminal rate	32/33 (97%)	31/31 (100%)	32/32 (100%)	24/24 (100%)
First incidence (days)	273	498	393	499
Poly-3 test	P = 0.309	P = 0.496	P = 0.496	P = 0.496

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal kill.
[d]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.

Table A-3. Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Male F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
β-Picoline (November 2004)	3/50	0/50	3/50
Sodium dichromate dihydrate (October 2002)	4/50	0/50	4/50
Total (%)	7/100 (7.0%)	0/100	7/100 (7.0%)
Mean ± standard deviation	7.0% ± 1.4%	–	7.0% ± 1.4%
Range	6%–8%	–	6%–8%
Overall Historical Incidence: All Routes			
Total (%)	31/1,249 (2.5%)	15/1,249 (1.2%)	45/1,249 (3.6%)
Mean ± standard deviation	2.5% ± 2.6%	1.2% ± 1.4%	3.6% ± 2.8%
Range	0%–8%	0%–6%	0%–10%

[a]Data as of May 18, 2011.

Table A-4. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	12	15	23
Natural deaths	8	7	3	3
Survivors				
Terminal kill	33	31	32	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Hyperplasia, squamous	–	1 (2%)	–	–
Inflammation, suppurative	1 (2%)	–	–	–
Intestine large, cecum	(45)	(47)	(48)	(48)
Inflammation, chronic active	–	–	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Intestine large, colon	(47)	(49)	(49)	(49)
Parasite metazoan	3 (6%)	4 (8%)	7 (14%)	1 (2%)
Intestine large, rectum	(47)	(49)	(49)	(50)
Infiltration cellular, mixed cell	–	1 (2%)	–	–
Parasite metazoan	2 (4%)	5 (10%)	3 (6%)	5 (10%)
Intestine small, duodenum	(48)	(49)	(49)	(50)
Intestine small, ileum	(46)	(47)	(48)	(49)
Inflammation, chronic active	–	1 (2%)	–	–
Intestine small, jejunum	(45)	(47)	(48)	(49)
Inflammation, chronic active	1 (2%)	–	–	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	–	1 (2%)	–
Atrophy	–	–	1 (2%)	–
Basophilic focus	15 (30%)	14 (28%)	16 (32%)	10 (20%)
Clear cell focus	13 (26%)	23 (46%)	20 (40%)	15 (30%)
Congestion	–	–	–	1 (2%)
Degeneration, cystic	3 (6%)	6 (12%)	7 (14%)	2 (4%)
Eosinophilic focus	7 (14%)	1 (2%)	2 (4%)	5 (10%)
Fatty change	7 (14%)	6 (12%)	7 (14%)	9 (18%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Fibrosis	–	–	1 (2%)	–
Hepatodiaphragmatic nodule	4 (8%)	3 (6%)	2 (4%)	6 (12%)
Inflammation, chronic active	–	3 (6%)	3 (6%)	1 (2%)
Mixed cell focus	6 (12%)	3 (6%)	6 (12%)	3 (6%)
Necrosis	2 (4%)	3 (6%)	–	2 (4%)
Vacuolization cytoplasmic	15 (30%)	12 (24%)	12 (24%)	16 (32%)
Bile duct, hyperplasia	40 (80%)	36 (72%)	44 (88%)	42 (84%)
Centrilobular, atrophy	–	1 (2%)	–	–
Centrilobular, necrosis	1 (2%)	–	1 (2%)	–
Periportal, fibrosis	–	1 (2%)	–	–
Periportal, inflammation	1 (2%)	–	1 (2%)	–
Vein, dilatation	–	1 (2%)	–	–
Mesentery	(4)	(8)	(4)	(6)
Fat, necrosis	1 (25%)	6 (75%)	3 (75%)	5 (83%)
Pancreas	(49)	(50)	(50)	(48)
Cyst	1 (2%)	–	2 (4%)	–
Hyperplasia	2 (4%)	–	–	1 (2%)
Infiltration cellular	–	1 (2%)	1 (2%)	–
Lipomatosis	–	–	–	1 (2%)
Acinus, atrophy	18 (37%)	31 (62%)	31 (62%)	25 (52%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	–	–	1 (2%)	–
Infiltration cellular	–	1 (2%)	–	–
Duct, inflammation	–	1 (2%)	–	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Edema	–	–	1 (2%)	–
Erosion	1 (2%)	–	–	–
Fibrosis	1 (2%)	6 (12%)	2 (4%)	1 (2%)
Hyperplasia, squamous	15 (30%)	18 (36%)	7 (14%)	17 (34%)
Inflammation	–	1 (2%)	–	2 (4%)
Ulcer	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Angiectasis	–	1 (2%)	–	–
Erosion	1 (2%)	3 (6%)	–	–
Fibrosis	1 (2%)	–	–	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Hyperplasia	1 (2%)	–	–	–
Inflammation, chronic active	4 (8%)	6 (12%)	4 (8%)	3 (6%)
Metaplasia, squamous	–	1 (2%)	–	–
Ulcer	–	–	–	2 (4%)
Tongue	(0)	(0)	(2)	(1)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization	–	1 (2%)	–	–
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	49 (98%)	50 (100%)	50 (100%)	49 (98%)
Mineralization	–	1 (2%)	–	–
Atrium, thrombosis	5 (10%)	5 (10%)	–	–
Epicardium, inflammation, chronic active	–	–	–	1 (2%)
Valve, inflammation, chronic active	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	–	–	2 (4%)
Hyperplasia	20 (40%)	31 (62%)	27 (54%)	21 (42%)
Necrosis	1 (2%)	–	1 (2%)	–
Vacuolization cytoplasmic	3 (6%)	–	–	1 (2%)
Adrenal medulla	(50)	(49)	(49)	(50)
Hyperplasia	12 (24%)	18 (37%)	13 (27%)	7 (14%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(50)	(47)	(48)	(48)
Hyperplasia	1 (2%)	–	–	–
Pituitary gland	(50)	(49)	(50)	(49)
Cyst	2 (4%)	–	2 (4%)	–
Metaplasia, osseous	1 (2%)	–	–	1 (2%)
Necrosis	–	–	–	1 (2%)
Pars distalis, hyperplasia	16 (32%)	11 (22%)	19 (38%)	12 (24%)
Pars intermedia, hyperplasia	–	–	1 (2%)	–
Pars nervosa, hyperplasia	–	–	1 (2%)	–
Thyroid gland	(50)	(50)	(50)	(50)
Congestion	1 (2%)	–	–	–
C-cell, hyperplasia	6 (12%)	4 (8%)	11 (22%)	7 (14%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Follicle, cyst	–	1 (2%)	–	1 (2%)
Follicular cell, hyperplasia	–	1 (2%)	1 (2%)	–
General Body System				
Peritoneum	(2)	(1)	(1)	(2)
Inflammation, chronic active	–	–	–	1 (50%)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Degeneration, fatty	–	1 (2%)	–	–
Inflammation, chronic active	–	–	2 (4%)	2 (4%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	–	1 (2%)	1 (2%)	–
Hyperplasia, squamous	–	1 (2%)	–	–
Inflammation	15 (30%)	10 (20%)	10 (20%)	13 (26%)
Prostate	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	–	1 (2%)
Hyperplasia, adenomatous	1 (2%)	–	–	1 (2%)
Inflammation	31 (63%)	45 (90%)	45 (90%)	35 (70%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	–	1 (2%)	–	–
Inflammation	–	–	2 (4%)	1 (2%)
Testes	(50)	(50)	(50)	(50)
Atrophy	3 (6%)	9 (18%)	5 (10%)	3 (6%)
Inflammation, chronic active	1 (2%)	–	–	–
Interstitial cell, hyperplasia	12 (24%)	18 (36%)	17 (34%)	13 (26%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(9)	(11)	(11)	(8)
Congestion	1 (11%)	–	–	–
Hyperplasia, lymphoid	1 (11%)	–	–	–
Deep cervical, ectasia	–	–	–	1 (13%)
Deep cervical, hemorrhage	–	1 (9%)	–	–
Deep cervical, infiltration cellular	–	–	–	1 (13%)
Mediastinal, congestion	1 (11%)	–	–	–
Mediastinal, ectasia	1 (11%)	1 (9%)	4 (36%)	3 (38%)
Mediastinal, hemorrhage	–	–	1 (9%)	–
Mediastinal, hyperplasia	1 (11%)	1 (9%)	1 (9%)	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Mediastinal, infiltration cellular, mixed cell	–	1 (9%)	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Congestion	1 (2%)	–	–	–
Ectasia	–	2 (4%)	2 (4%)	–
Inflammation, granulomatous	–	–	1 (2%)	–
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hyperplasia, lymphoid	–	1 (2%)	–	–
Inflammation, granulomatous	–	–	1 (2%)	–
Necrosis	–	–	–	1 (2%)
Capsule, hemorrhage	–	–	1 (2%)	–
Capsule, hyperplasia	–	–	–	1 (2%)
Lymphoid follicle, depletion cellular	–	1 (2%)	–	–
Lymphoid follicle, hyperplasia	1 (2%)	–	–	–
Thymus	(49)	(49)	(45)	(50)
Integumentary System				
Mammary gland	(50)	(49)	(50)	(50)
Galactocele	12 (24%)	7 (14%)	9 (18%)	12 (24%)
Hyperplasia	3 (6%)	6 (12%)	–	5 (10%)
Inflammation	–	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	–	–	–
Hyperplasia, squamous	2 (4%)	–	–	–
Inflammation, suppurative	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	1 (2%)	–	–
Subcutaneous tissue, infiltration cellular, mixed cell	–	1 (2%)	–	–
Subcutaneous tissue, inflammation, chronic active	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	1 (2%)	–	–	1 (2%)
Inflammation, chronic active	–	1 (2%)	–	–
Skeletal muscle	(0)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	5 (10%)	9 (18%)	8 (16%)	9 (18%)
Hemorrhage	1 (2%)	–	2 (4%)	3 (6%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body	–	–	–	1 (2%)
Inflammation, suppurative	1 (2%)	–	–	
Inflammation, granulomatous	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	12 (24%)	11 (22%)	12 (24%)	8 (16%)
Alveolar epithelium, metaplasia, squamous	–	1 (2%)	–	–
Perivascular, hemorrhage	–	–	1 (2%)	1 (2%)
Perivascular, inflammation, chronic active	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Edema	–	–	–	1 (2%)
Foreign body	10 (20%)	20 (40%)	15 (30%)	10 (20%)
Inflammation	21 (42%)	23 (46%)	16 (32%)	24 (48%)
Mineralization	–	1 (2%)	–	–
Necrosis	1 (2%)	–	–	–
Glands, cyst	–	–	–	1 (2%)
Nasolacrimal duct, foreign body	–	–	1 (2%)	–
Nasolacrimal duct, hyperplasia, squamous	–	–	1 (2%)	–
Nasolacrimal duct, inflammation	1 (2%)	–	1 (2%)	–
Olfactory epithelium, atrophy	–	–	–	1 (2%)
Olfactory epithelium, erosion	–	–	–	1 (2%)
Olfactory epithelium, metaplasia	–	–	1 (2%)	1 (2%)
Olfactory epithelium, necrosis	–	–	–	1 (2%)
Respiratory epithelium, degeneration, cystic	1 (2%)	–	–	
Respiratory epithelium, erosion	–	–	–	1 (2%)
Respiratory epithelium, hyperplasia	14 (28%)	20 (40%)	15 (30%)	17 (34%)
Respiratory epithelium, metaplasia, squamous	–	1 (2%)	–	–
Respiratory epithelium, ulcer	2 (4%)	–	–	–
Squamous epithelium, hyperplasia	–	1 (2%)	1 (2%)	
Trachea	(49)	(50)	(50)	(50)
Amyloid deposition	1 (2%)	–	–	
Inflammation	–	–	–	1 (2%)
Glands, cyst	2 (4%)	–	–	–
Special Senses System				
Ear	(2)	(0)	(0)	(0)
Cyst	1 (50%)	–	–	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Hyperplasia, squamous	1 (50%)	–	–	–
Eye	(50)	(50)	(50)	(50)
Cataract	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Cornea, hyperplasia, squamous	–	–	2 (4%)	–
Cornea, inflammation, chronic active	1 (2%)	–	–	1 (2%)
Retina, degeneration	–	1 (2%)	1 (2%)	1 (2%)
Sclera, inflammation, chronic active	1 (2%)	–	–	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	–	2 (4%)	–
Zymbal's gland	(1)	(0)	(1)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Accumulation, hyaline droplet	–	–	–	1 (2%)
Dilatation	–	1 (2%)	–	–
Infarct, acute	–	1 (2%)	1 (2%)	1 (2%)
Metaplasia, osseous	–	1 (2%)	–	–
Nephropathy	48 (96%)	50 (100%)	50 (100%)	50 (100%)
Medulla, renal tubule, necrosis	–	1 (2%)	–	–
Pelvis, inflammation, chronic active	–	1 (2%)	2 (4%)	–
Renal tubule, hyperplasia	–	1 (2%)	–	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage	–	1 (2%)	–	–
Infiltration cellular, mixed cell, chronic active	–	–	–	1 (2%)
Inflammation, suppurative	–	1 (2%)	–	–
Transitional epithelium, hyperplasia	–	1 (2%)	–	–

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix B. Summary of Lesions in Female Rats in the Two-year Drinking Water Study of β-Picoline

Tables

Table B-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	13	14	15
Natural deaths	5	5	3	5
Survivors				
Died last week of study	1	–	–	–
Terminal kill	29	32	33	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(46)	(45)	(47)	(45)
Intestine large, colon	(47)	(48)	(50)	(49)
Intestine large, rectum	(47)	(48)	(50)	(49)
Leiomyosarcoma, metastatic, uterus	1 (2%)	–	–	–
Sarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Intestine small, duodenum	(50)	(47)	(50)	(50)
Intestine small, ileum	(47)	(46)	(49)	(50)
Intestine small, jejunum	(48)	(46)	(47)	(48)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma	2 (4%)	–	–	–
Mesentery	(4)	(7)	(7)	(8)
Sarcoma, metastatic, uncertain primary site	–	–	1 (14%)	–
Oral mucosa	(0)	(0)	(1)	(2)
Squamous cell papilloma	–	–	1 (100%)	1 (50%)
Pancreas	(50)	(50)	(49)	(50)
Sarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(49)	(50)
Tongue	(0)	(0)	(0)	(1)
Squamous cell papilloma	–	–	–	1 (100%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	2 (4%)	–	3 (6%)	–
Pheochromocytoma malignant	–	–	–	1 (2%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma	–	1 (2%)	–	1 (2%)
Parathyroid gland	(47)	(49)	(48)	(48)
Pituitary gland	(50)	(49)	(49)	(50)
Pars distalis, adenoma	30 (60%)	27 (55%)	29 (59%)	26 (52%)
Pars nervosa, craniopharyngioma	–	–	–	1 (2%)
Thyroid gland	(50)	(48)	(50)	(50)
Carcinoma	–	–	1 (2%)	1 (2%)
Bilateral, c-cell, adenoma	1 (2%)	–	2 (4%)	–
C-cell, adenoma	9 (18%)	3 (6%)	1 (2%)	6 (12%)
C-cell, carcinoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Follicular cell, adenoma	–	–	–	1 (2%)
General Body System				
Peritoneum	(0)	(1)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	7 (14%)	12 (24%)	8 (16%)
Carcinoma	–	1 (2%)	1 (2%)	2 (4%)
Sarcoma	1 (2%)	–	1 (2%)	–
Bilateral, adenoma	–	2 (4%)	–	–
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor benign	–	–	1 (2%)	–
Granulosa cell tumor malignant	–	2 (4%)	–	–
Granulosa-theca tumor malignant	–	–	–	1 (2%)
Schwannoma malignant	1 (2%)	–	–	–
Uterus	(50)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)	–	1 (2%)	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Polyp stromal	8 (16%)	13 (26%)	13 (26%)	6 (12%)
Cervix, sarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Endometrium, adenoma	–	–	1 (2%)	–
Endometrium, carcinoma	1 (2%)	–	–	–
Vagina	(1)	(1)	(0)	(2)
Polyp	1 (100%)	1 (100%)	–	2 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(5)	(7)	(5)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(49)	(50)	(50)	(47)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	–	–	2 (4%)	1 (2%)
Carcinoma	2 (4%)	–	1 (2%)	–
Fibroadenoma	22 (44%)	15 (30%)	17 (34%)	17 (34%)
Fibroadenoma, multiple	12 (24%)	16 (32%)	11 (22%)	6 (12%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	–	–	–
Keratoacanthoma	–	–	–	1 (2%)
Subcutaneous tissue, fibroma	2 (4%)	2 (4%)	1 (2%)	–
Subcutaneous tissue, fibrosarcoma	–	1 (2%)	1 (2%)	–
Subcutaneous tissue, hemangiosarcoma	–	–	1 (2%)	–
Subcutaneous tissue, lipoma	–	–	–	1 (2%)
Subcutaneous tissue, sarcoma	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	–	–	–	1 (2%)
Skeletal muscle	(0)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Glioma benign	1 (2%)	–	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	–	3 (6%)	2 (4%)	5 (10%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Alveolar/bronchiolar carcinoma	–	1 (2%)	–	–
Carcinoma, metastatic, thyroid gland	1 (2%)	–	1 (2%)	1 (2%)
Hemangiosarcoma, metastatic, skin	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Respiratory epithelium, adenoma	–	–	1 (2%)	–
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(48)	(48)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(2)	(1)
Carcinoma	–	–	2 (100%)	–
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Renal tubule, adenoma	–	1 (2%)	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Leiomyosarcoma, metastatic, uterus	1 (2%)	–	–	–
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	–	1 (2%)	1 (2%)
Leukemia mononuclear	12 (24%)	15 (30%)	18 (36%)	10 (20%)
Lymphoma malignant	–	–	–	1 (2%)
Mesothelioma malignant	–	1 (2%)	–	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	49	48	45
Total primary neoplasms	119	117	130	107
Total animals with benign neoplasms	47	45	44	43
Total benign neoplasms	100	95	100	87
Total animals with malignant neoplasms	17	18	26	18
Total malignant neoplasms	19	22	30	20
Total animals with metastatic neoplasms	2	–	3	2
Total metastatic neoplasms	3	–	6	2
Total animals with malignant neoplasms of uncertain primary site	–	–	1	1

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table B-2. Statistical Analysis of Primary Neoplasms in Female Rats in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Adrenal Cortex: Adenoma				
Overall rate[a]	2/50 (4%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate[b]	4.6%	9.2%	4.4%	6.9%
Terminal rate[c]	1/30 (3%)	4/32 (13%)	1/33 (3%)	2/30 (7%)
First incidence (days)	691	727 (T)	666	656
Poly-3 test[d]	P = 0.518	P = 0.334	P = 0.681N	P = 0.495
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	0/50 (0%)
Adjusted rate	4.6%	0.0%	6.6%	0%
Terminal rate	2/30 (7%)	0/32 (0%)	2/33 (6%)	0/30 (0%)
First incidence (days)	727 (T)	–[e]	683	–
Poly-3 test	P = 0.299N	P = 0.237N	P = 0.518	P = 0.240N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.6%	0.0%	6.6%	2.3%
Terminal rate	2/30 (7%)	0/32 (0%)	2/33 (6%)	1/30 (3%)
First incidence (days)	727 (T)	–	683	727 (T)
Poly-3 test	P = 0.560N	P = 0.237N	P = 0.518	P = 0.505N
Clitoral Gland: Adenoma				
Overall rate	7/50 (14%)	9/50 (18%)	12/50 (24%)	8/50 (16%)
Adjusted rate	16.0%	20.1%	26.5%	18.2%
Terminal rate	6/30 (20%)	7/32 (22%)	11/33 (33%)	5/30 (17%)
First incidence (days)	691	474	721	515
Poly-3 test	P = 0.452	P = 0.408	P = 0.168	P = 0.505
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	10/50 (20%)	12/50 (24%)	10/50 (20%)
Adjusted rate	16.0%	22.4%	26.5%	22.7%
Terminal rate	6/30 (20%)	8/32 (25%)	11/33 (33%)	6/30 (20%)
First incidence (days)	691	474	721	515
Poly-3 test	P = 0.283	P = 0.311	P = 0.168	P = 0.299
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	6.7%	4.4%	11.5%
Terminal rate	0/30 (0%)	1/32 (3%)	2/33 (6%)	4/30 (13%)
First incidence (days)	–	526	727 (T)	638
Poly-3 test	P = 0.029	P = 0.122	P = 0.245	P = 0.030

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	0/50 (0%)	4/50 (8%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	9.0%	4.4%	11.5%
Terminal rate	0/30 (0%)	2/32 (6%)	2/33 (6%)	4/30 (13%)
First incidence (days)	–	526	727 (T)	638
Poly-3 test	P = 0.050	P = 0.063	P = 0.245	P = 0.030
Mammary Gland: Fibroadenoma				
Overall rate	34/50 (68%)	31/50 (62%)	28/50 (56%)	23/50 (46%)
Adjusted rate	74.6%	67.8%	60.4%	50.2%
Terminal rate	23/30 (77%)	23/32 (72%)	22/33 (67%)	13/30 (43%)
First incidence (days)	579	526	642	540
Poly-3 test	P = 0.006N	P = 0.306N	P = 0.100N	P = 0.010N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	34/50 (68%)[f]	31/50 (62%)	29/50 (58%)[g]	24/50 (48%)
Adjusted rate	74.6%	67.8%	62.4%	51.6%
Terminal rate	23/30 (77%)	23/32 (72%)	22/33 (67%)	13/30 (43%)
First incidence (days)	579	526	642	474
Poly-3 test	P = 0.009N	P = 0.306N	P = 0.140N	P = 0.014N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	4.6%	0.0%	6.6%	2.3%
Terminal rate	2/30 (7%)	0/32 (0%)	1/33 (3%)	0/30 (0%)
First incidence (days)	727 (T)	–	642	474
Poly-3 test	P = 0.553N	P = 0.237N	P = 0.521	P = 0.499N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	30/50 (60%)	27/49 (55%)	29/49 (59%)	26/50 (52%)
Adjusted rate	62.1%	59.6%	63.0%	55.8%
Terminal rate	15/30 (50%)	19/32 (59%)	23/33 (70%)	14/30 (47%)
First incidence (days)	454	474	527	474
Poly-3 test	P = 0.316N	P = 0.488N	P = 0.546	P = 0.339N
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.6%	6.9%	4.4%	2.3%
Terminal rate	1/30 (3%)	3/32 (9%)	1/33 (3%)	1/30 (3%)
First incidence (days)	712	727 (T)	579	727 (T)
Poly-3 test	P = 0.316N	P = 0.498	P = 0.678N	P = 0.505N

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Thyroid Gland (C-Cell): Adenoma				
Overall rate	10/50 (20%)	3/48 (6%)	3/50 (6%)	6/50 (12%)
Adjusted rate	22.5%	7.1%	6.6%	13.7%
Terminal rate	9/30 (30%)	3/32 (9%)	2/33 (6%)	4/30 (13%)
First incidence (days)	488	727 (T)	666	638
Poly-3 test	P = 0.236N	P = 0.040N	P = 0.030N	P = 0.213N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/50 (22%)	4/48 (8%)	5/50 (10%)	7/50 (14%)
Adjusted rate	24.8%	9.4%	11.0%	16.0%
Terminal rate	10/30 (33%)	4/32 (13%)	4/33 (12%)	5/30 (17%)
First incidence (days)	488	727 (T)	666	638
Poly-3 test	P = 0.268N	P = 0.051N	P = 0.074N	P = 0.224N
Uterus: Stromal Polyp				
Overall rate	8/50 (16%)	13/50 (26%)	13/50 (26%)	6/50 (12%)
Adjusted rate	18.3%	29.1%	28.1%	13.8%
Terminal rate	8/30 (27%)	10/32 (31%)	8/33 (24%)	5/30 (17%)
First incidence (days)	727 (T)	526	642	607
Poly-3 test	P = 0.235N	P = 0.173	P = 0.199	P = 0.388N
All Organs: Mononuclear Leukemia				
Overall rate	12/50 (24%)	15/50 (30%)	18/50 (36%)	10/50 (20%)
Adjusted rate	26.3%	33.1%	38.4%	22.3%
Terminal rate	5/30 (17%)	7/32 (22%)	10/33 (30%)	6/30 (20%)
First incidence (days)	454	614	527	515
Poly-3 test	P = 0.336N	P = 0.314	P = 0.153	P = 0.420N
All Organs: Benign Tumors				
Overall rate	47/50 (94%)	45/50 (90%)	44/50 (88%)	43/50 (86%)
Adjusted rate	96.0%	93.9%	91.4%	88.2%
Terminal rate	29/30 (97%)	32/32 (100%)	32/33 (97%)	26/30 (87%)
First incidence (days)	454	474	527	474
Poly-3 test	P = 0.067N	P = 0.487N	P = 0.278N	P = 0.125N
All Organs: Malignant Tumors				
Overall rate	17/50 (34%)	18/50 (36%)	27/50 (54%)	18/50 (36%)
Adjusted rate	36.5%	39.2%	56.1%	39.4%
Terminal rate	8/30 (27%)	9/32 (28%)	15/33 (46%)	11/30 (37%)
First incidence (days)	454	488	527	515
Poly-3 test	P = 0.354	P = 0.478	P = 0.040	P = 0.470

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
All Organs: Benign or Malignant Tumors				
Overall rate	49/50 (98%)	49/50 (98%)	48/50 (96%)	45/50 (90%)
Adjusted rate	98.0%	99.2%	98.1%	91.1%
Terminal rate	29/30 (97%)	32/32 (100%)	33/33 (100%)	27/30 (90%)
First incidence (days)	454	474	527	474
Poly-3 test	P = 0.019N*	P = 0.644	P = 0.787	P = 0.132N

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal kill.
[d]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.
[f]A single incidence of carcinoma occurred in each of two animals that also had fibroadenoma.
[g]A single incidence of carcinoma occurred in an animal that also had fibroadenoma.

Table B-3. Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Female F344/N Rats[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
β-Picoline (November 2004)	0/50	0/50	0/50
Sodium dichromate dihydrate (October 2002)	4/50	0/50	4/50
Total (%)	4/100 (4.0%)	0/100	4/100 (4.0%)
Mean ± standard deviation	4.0% ± 5.7%	–	4.0% ± 5.7%
Range	0%–8%	–	0%–8%
Overall Historical Incidence: All Routes			
Total (%)	25/1,200 (2.1%)	3/1,200 (0.3%)	27/1,200 (2.3%)
Mean ± standard deviation	2.1% ± 2.9%	0.3% ± 0.7%	2.3% ± 2.9%
Range	0%–8%	0%–2%	0%–8%

[a]Data as of May 18, 2011.

Table B-4. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	13	14	15
Natural deaths	5	5	3	5
Survivors				
Died last week of study	1	–	–	–
Terminal kill	29	32	33	30
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(46)	(45)	(47)	(45)
Inflammation, chronic active	1 (2%)	–	–	–
Intestine large, colon	(47)	(48)	(50)	(49)
Inflammation, chronic active	–	1 (2%)	–	–
Parasite metazoan	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Intestine large, rectum	(47)	(48)	(50)	(49)
Inflammation, chronic active	–	1 (2%)	1 (2%)	–
Parasite metazoan	3 (6%)	8 (17%)	1 (2%)	7 (14%)
Intestine small, duodenum	(50)	(47)	(50)	(50)
Intestine small, ileum	(47)	(46)	(49)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	–
Parasite metazoan	–	–	–	1 (2%)
Intestine small, jejunum	(48)	(46)	(47)	(48)
Inflammation, chronic active	1 (2%)	–	2 (4%)	1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	–	3 (6%)	1 (2%)	–
Atypia cellular	–	–	1 (2%)	–
Basophilic focus	37 (74%)	41 (82%)	46 (92%)	45 (90%)
Clear cell focus	10 (20%)	12 (24%)	12 (24%)	10 (20%)
Congestion	1 (2%)	1 (2%)	–	1 (2%)
Degeneration, cystic	1 (2%)	–	–	–
Eosinophilic focus	12 (24%)	14 (28%)	12 (24%)	13 (26%)
Fatty change	8 (16%)	7 (14%)	8 (16%)	11 (22%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Fatty change, focal	2 (4%)	13 (26%)	7 (14%)	8 (16%)
Hematopoietic cell proliferation	1 (2%)	–	–	–
Hepatodiaphragmatic nodule	2 (4%)	7 (14%)	3 (6%)	1 (2%)
Inflammation, chronic active	2 (4%)	4 (8%)	3 (6%)	–
Mixed cell focus	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Necrosis	1 (2%)	–	2 (4%)	–
Regeneration	1 (2%)	–	–	–
Vacuolization cytoplasmic	4 (8%)	1 (2%)	2 (4%)	–
Bile duct, hyperplasia	10 (20%)	2 (4%)	9 (18%)	7 (14%)
Serosa, fibrosis	–	–	1 (2%)	2 (4%)
Vein, dilatation	1 (2%)	–	–	1 (2%)
Vein, infiltration cellular, mixed cell	–	1 (2%)	–	–
Mesentery	(4)	(7)	(7)	(8)
Fibrosis	–	–	–	1 (13%)
Fat, necrosis	4 (100%)	6 (86%)	6 (86%)	7 (88%)
Oral mucosa	(0)	(0)	(1)	(2)
Gingival, foreign body	–	–	1 (100%)	1 (50%)
Gingival, inflammation, chronic active	–	–	1 (100%)	–
Pancreas	(50)	(50)	(49)	(50)
Atrophy	–	–	1 (2%)	1 (2%)
Basophilic focus	1 (2%)	–	2 (4%)	–
Cyst	1 (2%)	2 (4%)	–	–
Infiltration cellular	2 (4%)	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	1 (2%)	–
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	–	–	1 (2%)	–
Fibrosis	4 (8%)	1 (2%)	4 (8%)	1 (2%)
Hyperplasia, basal cell, focal	–	–	1 (2%)	–
Hyperplasia, squamous	7 (14%)	5 (10%)	6 (12%)	7 (14%)
Inflammation	1 (2%)	–	1 (2%)	–
Ulcer	1 (2%)	2 (4%)	3 (6%)	–
Stomach, glandular	(50)	(50)	(49)	(50)
Inflammation, chronic active	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Mineralization	–	–	–	2 (4%)
Ulcer	1 (2%)	–	–	–

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Tongue	(0)	(0)	(0)	(1)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, mineralization	–	–	–	1 (2%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	48 (96%)	50 (100%)	50 (100%)	49 (98%)
Mineralization	–	–	–	2 (4%)
Artery, infiltration cellular, mononuclear cell	1 (2%)	–	–	–
Atrium, thrombosis	1 (2%)	1 (2%)	–	–
Endocardium, hyperplasia	–	–	1 (2%)	–
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	1 (2%)	–	1 (2%)
Amyloid deposition	–	1 (2%)	–	–
Hyperplasia	26 (52%)	25 (50%)	34 (68%)	22 (44%)
Necrosis	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Vacuolization cytoplasmic	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	3 (6%)	1 (2%)	3 (6%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	–	–	–	1 (2%)
Parathyroid gland	(47)	(49)	(48)	(48)
Hyperplasia	–	–	–	1 (2%)
Pituitary gland	(50)	(49)	(49)	(50)
Angiectasis	–	1 (2%)	–	–
Cyst	–	–	1 (2%)	–
Pars distalis, cyst	1 (2%)	1 (2%)	–	1 (2%)
Pars distalis, hyperplasia	19 (38%)	19 (39%)	20 (41%)	20 (40%)
Pars intermedia, hyperplasia	1 (2%)	–	–	–
Thyroid gland	(50)	(48)	(50)	(50)
Mineralization	–	–	–	1 (2%)
C-cell, hyperplasia	8 (16%)	6 (13%)	5 (10%)	7 (14%)
Follicle, cyst	–	1 (2%)	1 (2%)	–
General Body System				
Peritoneum	(0)	(1)	(0)	(0)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Fibrosis	1 (2%)	–	1 (2%)	1 (2%)
Hyperplasia	2 (4%)	4 (8%)	3 (6%)	4 (8%)
Inflammation	9 (18%)	5 (10%)	6 (12%)	6 (12%)
Duct, dilatation	–	1 (2%)	–	–
Ovary	(50)	(50)	(50)	(50)
Atrophy	–	1 (2%)	–	1 (2%)
Cyst	4 (8%)	3 (6%)	–	3 (6%)
Uterus	(50)	(50)	(50)	(50)
Hemorrhage	–	1 (2%)	–	–
Inflammation, suppurative	–	1 (2%)	–	–
Cervix, cyst	4 (8%)	2 (4%)	3 (6%)	3 (6%)
Endometrium, hyperplasia, cystic	1 (2%)	8 (16%)	7 (14%)	8 (16%)
Vagina	(1)	(1)	(0)	(2)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Fibrosis	–	1 (2%)	–	–
Lymph node	(5)	(7)	(5)	(2)
Deep cervical, ectasia	–	–	1 (20%)	–
Deep cervical, hemorrhage	–	1 (14%)	–	–
Deep cervical, infiltration cellular, histiocyte	–	–	1 (20%)	–
Mediastinal, ectasia	–	1 (14%)	–	–
Mediastinal, hemorrhage	–	1 (14%)	–	1 (50%)
Mediastinal, hyperplasia	–	1 (14%)	–	–
Mediastinal, infiltration cellular, histiocyte	–	–	–	1 (50%)
Mediastinal, infiltration cellular, mixed cell	–	–	1 (20%)	–
Pancreatic, ectasia	1 (20%)	–	–	–
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	–	–	1 (2%)	–
Infiltration cellular, mast cell	–	–	1 (2%)	–
Infiltration cellular, mononuclear cell	–	–	1 (2%)	–
Infiltration cellular, plasma cell	–	–	–	1 (2%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Spleen	(50)	(50)	(50)	(50)
Angiectasis	–	–	1 (2%)	–
Atrophy	–	–	1 (2%)	2 (4%)
Degeneration, cystic, focal	–	–	1 (2%)	–
Hyperplasia, lymphoid	4 (8%)	2 (4%)	1 (2%)	–
Necrosis	–	1 (2%)	–	1 (2%)
Thymus	(49)	(50)	(50)	(47)
Cyst	1 (2%)	–	–	1 (2%)
Fibrosis	–	–	–	1 (2%)
Hyperplasia, lymphoid	1 (2%)	–	–	–
Inflammation, suppurative	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Galactocele	12 (24%)	8 (16%)	3 (6%)	4 (8%)
Hyperplasia	4 (8%)	4 (8%)	6 (12%)	4 (8%)
Inflammation	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	–	1 (2%)	–
Hyperplasia, squamous	–	–	1 (2%)	–
Inflammation, chronic active	–	1 (2%)	–	–
Ulcer	–	2 (4%)	2 (4%)	1 (2%)
Lip, inflammation, suppurative	–	–	–	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cyst	–	–	–	1 (2%)
Hyperostosis	1 (2%)	–	–	–
Inflammation, chronic active	1 (2%)	–	–	1 (2%)
Skeletal muscle	(0)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	13 (26%)	7 (14%)	7 (14%)	9 (18%)
Hemorrhage	–	2 (4%)	–	1 (2%)
Inflammation, chronic active	–	1 (2%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Fibrosis	–	–	–	1 (2%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Foreign body	1 (2%)	–	–	–
Inflammation, suppurative	–	–	1 (2%)	–
Inflammation, granulomatous	1 (2%)	–	1 (2%)	–
Inflammation, chronic active	1 (2%)	–	–	–
Metaplasia, squamous	1 (2%)	–	–	–
Pigmentation	1 (2%)	–	–	–
Thrombosis	–	–	–	1 (2%)
Alveolar epithelium, hyperplasia	7 (14%)	14 (28%)	14 (28%)	11 (22%)
Alveolar epithelium, metaplasia, squamous	–	3 (6%)	1 (2%)	1 (2%)
Bronchiole, inflammation, suppurative	–	–	1 (2%)	–
Bronchiole, inflammation, chronic active	–	–	2 (4%)	–
Serosa, fibrosis	–	1 (2%)	–	2 (4%)
Serosa, inflammation, granulomatous	–	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Foreign body	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Inflammation	3 (6%)	5 (10%)	4 (8%)	3 (6%)
Glands, hyperplasia	–	1 (2%)	–	–
Nasolacrimal duct, foreign body	1 (2%)	–	–	–
Nasolacrimal duct, inflammation	2 (4%)	–	–	–
Olfactory epithelium, atrophy	–	1 (2%)	–	–
Olfactory epithelium, metaplasia	–	1 (2%)	–	–
Respiratory epithelium, hyperplasia	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Turbinate, cyst	–	–	–	2 (4%)
Trachea	(50)	(50)	(50)	(50)
Infiltration cellular, mixed cell	–	1 (2%)	–	–
Special Senses System				
Eye	(50)	(48)	(48)	(50)
Atrophy	1 (2%)	–	–	–
Cataract	8 (16%)	13 (27%)	14 (29%)	10 (20%)
Anterior chamber, inflammation, suppurative	–	1 (2%)	–	1 (2%)
Cornea, edema	–	–	1 (2%)	–
Cornea, hyperplasia	–	1 (2%)	–	–
Cornea, inflammation, chronic active	2 (4%)	–	1 (2%)	–
Retina, degeneration	1 (2%)	2 (4%)	4 (8%)	3 (6%)

	0 mg/L	156.25 mg/L	312.5 mg/L	625 mg/L
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia	–	–	2 (4%)	1 (2%)
Inflammation, granulomatous	1 (2%)	–	–	–
Inflammation, chronic active	2 (4%)	1 (2%)	–	–
Zymbal's gland	(0)	(0)	(2)	(1)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst	–	–	2 (4%)	–
Degeneration, fatty	1 (2%)	–	–	–
Dilatation	–	–	–	1 (2%)
Fibrosis	8 (16%)	7 (14%)	8 (16%)	10 (20%)
Infarct	7 (14%)	8 (16%)	4 (8%)	5 (10%)
Mineralization	–	–	–	2 (4%)
Nephropathy	47 (96%)	50 (100%)	48 (96%)	50 (100%)
Papilla, hemorrhage	1 (2%)	–	5 (10%)	1 (2%)
Pelvis, inflammation, chronic active	8 (16%)	9 (18%)	8 (16%)	10 (20%)
Pelvis, metaplasia, squamous	–	–	1 (2%)	–
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, chronic active	–	–	1 (2%)	–
Transitional epithelium, hyperplasia	–	–	1 (2%)	–

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix C. Summary of Lesions in Male Mice in the Two-year Drinking Water Study of β-Picoline

Tables

Table C-1. Summary of the Incidence of Neoplasms in Male Mice in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	4	8	7
Natural deaths	20	20	15	10
Survivors				
Terminal kill	24	26	27	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(36)	(33)	(39)	(42)
Intestine large, cecum	(35)	(34)	(36)	(40)
Intestine large, colon	(40)	(40)	(41)	(40)
Sarcoma, metastatic, liver	–	1 (3%)	–	–
Intestine large, rectum	(39)	(38)	(42)	(41)
Intestine small, duodenum	(36)	(31)	(35)	(40)
Adenoma	–	–	–	1 (3%)
Intestine small, ileum	(36)	(35)	(39)	(40)
Intestine small, jejunum	(35)	(32)	(37)	(40)
Adenoma	–	1 (3%)	–	1 (3%)
Carcinoma	2 (6%)	1 (3%)	–	–
Liver	(50)	(50)	(50)	(50)
Cholangioma, multiple	–	–	–	1 (2%)
Hemangioma	–	1 (2%)	–	–
Hemangiosarcoma	5 (10%)	4 (8%)	3 (6%)	1 (2%)
Hepatoblastoma	3 (6%)	5 (10%)	9 (18%)	4 (8%)
Hepatoblastoma, multiple	3 (6%)	1 (2%)	2 (4%)	–
Hepatocellular adenoma	12 (24%)	11 (22%)	11 (22%)	15 (30%)
Hepatocellular adenoma, multiple	21 (42%)	30 (60%)	29 (58%)	20 (40%)
Hepatocellular carcinoma	20 (40%)	13 (26%)	17 (34%)	15 (30%)
Hepatocellular carcinoma, multiple	6 (12%)	10 (20%)	9 (18%)	8 (16%)
Hepatocholangiocarcinoma	2 (4%)	2 (4%)	1 (2%)	–
Ito cell tumor malignant	–	1 (2%)	–	–
Sarcoma	–	1 (2%)	–	–

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Mesentery	(7)	(1)	(1)	(1)
Hepatocholangiocarcinoma, metastatic, liver	1 (14%)	–	–	–
Pancreas	(49)	(48)	(48)	(49)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(47)	(47)	(47)	(47)
Hemangioma	–	1 (2%)	–	–
Squamous cell carcinoma	–	–	1 (2%)	–
Squamous cell papilloma	–	1 (2%)	–	3 (6%)
Stomach, glandular	(42)	(45)	(43)	(45)
Tooth	(35)	(41)	(42)	(41)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	–	–
Hepatocellular carcinoma, metastatic, liver	–	2 (4%)	2 (4%)	–
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Endocrine System				
Adrenal cortex	(49)	(48)	(50)	(48)
Adenoma	–	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	–	–	1 (2%)	–
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Subcapsular, adenoma	2 (4%)	2 (4%)	5 (10%)	2 (4%)
Subcapsular, carcinoma	–	–	–	2 (4%)
Adrenal medulla	(49)	(47)	(50)	(48)
Hepatocellular carcinoma, metastatic, liver	–	–	1 (2%)	–
Islets, pancreatic	(49)	(48)	(48)	(49)
Adenoma	1 (2%)	–	1 (2%)	–
Parathyroid gland	(37)	(39)	(44)	(31)
Pituitary gland	(47)	(49)	(49)	(49)
Pars distalis, adenoma	–	–	1 (2%)	–
Thyroid gland	(49)	(50)	(49)	(49)
Follicular cell, adenoma	1 (2%)	–	–	–
Follicular cell, carcinoma	–	1 (2%)	–	–

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(2)	(0)	(0)	(0)
Epididymis	(50)	(50)	(50)	(49)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(48)	(50)
Seminal vesicle	(47)	(48)	(49)	(47)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	–	–	–
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	–	1 (2%)	1 (2%)	1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(48)	(50)
Hemangiosarcoma	2 (4%)	–	–	2 (4%)
Lymph node	(2)	(1)	(0)	(2)
Mediastinal, hemangiosarcoma	–	–	–	1 (50%)
Mediastinal, hepatoblastoma, metastatic, liver	–	1 (100%)	–	–
Mediastinal, hepatocellular carcinoma,	–	–	–	1 (50%)
Metastatic, liver	–	–	–	–
Mediastinal, hepatocholangiocarcinoma, metastatic, liver	1 (50%)	–	–	–
Lymph node, mandibular	(50)	(49)	(49)	(50)
Carcinoma, metastatic, harderian gland	–	1 (2%)	–	–
Hepatocellular carcinoma, metastatic, liver	–	–	–	1 (2%)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)	–	–	–
Lymph node, mesenteric	(45)	(45)	(43)	(45)
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Spleen	(46)	(48)	(47)	(46)
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Thymus	(41)	(42)	(48)	(47)
Hemangiosarcoma, metastatic, liver	–	1 (2%)	–	–
Hepatoblastoma, metastatic, liver	–	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)	–	–
Sarcoma, metastatic, liver	–	1 (2%)	–	–

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)	2 (4%)	–	1 (2%)
Subcutaneous tissue, hemangioma	1 (2%)	–	–	1 (2%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)	–	2 (4%)	1 (2%)
Subcutaneous tissue, lipoma	–	–	1 (2%)	1 (2%)
Subcutaneous tissue, sarcoma	–	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chondrosarcoma	–	–	1 (2%)	–
Skeletal muscle	(1)	(0)	(0)	(0)
Rhabdomyosarcoma	1 (100%)	–	–	–
Nervous System				
Brain	(50)	(50)	(50)	(50)
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	6 (12%)	11 (22%)	14 (28%)	6 (12%)
Alveolar/bronchiolar adenoma, multiple	–	–	2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma	9 (18%)	7 (14%)	8 (16%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple	–	2 (4%)	–	3 (6%)
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	1 (2%)
Hemangiosarcoma	2 (4%)	–	–	–
Hemangiosarcoma, metastatic, liver	–	1 (2%)	–	–
Hepatoblastoma, metastatic, liver	2 (4%)	–	1 (2%)	–
Hepatocellular carcinoma, metastatic, liver	14 (28%)	12 (24%)	12 (24%)	6 (12%)
Hepatocholangiocarcinoma, metastatic, liver	1 (2%)	1 (2%)	1 (2%)	–
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)	–	–	–
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Sarcoma, metastatic, uncertain primary site	1 (2%)	–	–	–
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland	–	–	–	1 (2%)
Hemangiosarcoma	1 (2%)	–	–	–
Pleura	(0)	(1)	(0)	(1)
Hepatocellular carcinoma, metastatic, liver	–	1 (100%)	–	1 (100%)
Trachea	(47)	(49)	(45)	(49)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Special Senses System				
Eye	(36)	(39)	(43)	(40)
Harderian gland	(46)	(47)	(46)	(47)
Adenoma	5 (11%)	5 (11%)	3 (7%)	2 (4%)
Carcinoma	2 (4%)	4 (9%)	1 (2%)	3 (6%)
Rhabdomyosarcoma, metastatic, skeletal muscle	1 (2%)	–	–	–
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	–	2 (4%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver	–	–	1 (2%)	–
Sarcoma, metastatic, liver	–	1 (2%)	–	–
Renal tubule, adenoma, multiple	–	–	–	1 (2%)
Renal tubule, carcinoma	–	–	1 (2%)	–
Urethra	(3)	(3)	(5)	(3)
Urinary bladder	(45)	(45)	(45)	(46)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	–	1 (2%)	–	–
Lymphoma malignant	–	3 (6%)	2 (4%)	–
Mesothelioma benign	1 (2%)	–	–	–
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	50	48	46
Total primary neoplasms	112	124	128	105
Total animals with benign neoplasms	36	44	44	43
Total benign neoplasms	49	63	69	58
Total animals with malignant neoplasms	36	40	38	32
Total malignant neoplasms	62	61	59	47
Total animals with metastatic neoplasms	19	16	14	7
Total metastatic neoplasms	32	31	23	15
Total animals with malignant neoplasms of uncertain primary site	1	–	–	–

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table C-2. Statistical Analysis of Primary Neoplasms in Male Mice in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Adrenal Cortex: Adenoma				
Overall rate[a]	2/49 (4%)	1/48 (2%)	6/50 (12%)	3/48 (6%)
Adjusted rate[b]	5.4%	2.6%	14.7%	7.2%
Terminal rate[c]	1/24 (4%)	1/26 (4%)	5/27 (19%)	3/33 (9%)
First incidence (days)	543	730 (T)	685	730 (T)
Poly-3 test[d]	P = 0.344	P = 0.479N	P = 0.166	P = 0.558
Harderian Gland: Adenoma				
Overall rate	5/50 (10%)	5/50 (10%)	3/50 (6%)	2/50 (4%)
Adjusted rate	13.1%	12.2%	7.4%	4.6%
Terminal rate	2/24 (8%)	4/26 (15%)	2/27 (7%)	2/33 (6%)
First incidence (days)	562	722	707	730 (T)
Poly-3 test	P = 0.093N	P = 0.588N	P = 0.319N	P = 0.163N
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rate	5.3%	9.7%	2.5%	6.7%
Terminal rate	1/24 (4%)	2/26 (8%)	1/27 (4%)	1/33 (3%)
First incidence (days)	505	583	730 (T)	534
Poly-3 test	P = 0.561N	P = 0.383	P = 0.472N	P = 0.581
Harderian Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	9/50 (18%)	3/50 (6%)	5/50 (10%)
Adjusted rate	18.0%	21.7%	7.4%	11.1%
Terminal rate	3/24 (13%)	6/26 (23%)	2/27 (7%)	3/33 (9%)
First incidence (days)	505	583	707	534
Poly-3 test	P = 0.127N	P = 0.447	P = 0.135N	P = 0.280N
Liver: Hemangiosarcoma				
Overall rate	5/50 (10%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	12.9%	9.7%	7.3%	2.3%
Terminal rate	1/24 (4%)	1/26 (4%)	2/27 (7%)	0/33 (0%)
First incidence (days)	402	583	631	709
Poly-3 test	P = 0.048N	P = 0.456N	P = 0.323N	P = 0.074N
Liver: Hepatocellular Adenoma				
Overall rate	33/50 (66%)	41/50 (82%)	40/50 (80%)	35/50 (70%)
Adjusted rate	77.5%	84.9%	85.3%	73.7%
Terminal rate	22/24 (92%)	22/26 (85%)	24/27 (89%)	24/33 (73%)
First incidence (days)	460	407	470	465
Poly-3 test	P = 0.227N	P = 0.242	P = 0.225	P = 0.425N

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Liver: Hepatocellular Carcinoma				
Overall rate	26/50 (52%)	23/50 (46%)	26/50 (52%)	23/50 (46%)
Adjusted rate	59.2%	50.9%	56.1%	49.8%
Terminal rate	11/24 (46%)	11/26 (42%)	12/27 (44%)	13/33 (39%)
First incidence (days)	455	580	456	534
Poly-3 test	P = 0.268N	P = 0.277N	P = 0.465N	P = 0.242N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	45/50 (90%)	46/50 (92%)	48/50 (96%)	42/50 (84%)
Adjusted rate	96.2%	92.7%	98.4%	88.0%
Terminal rate	24/24 (100%)	24/26 (92%)	27/27 (100%)	28/33 (85%)
First incidence (days)	455	407	456	465
Poly-3 test	P = 0.084N	P = 0.374N	P = 0.468	P = 0.114N
Liver: Hepatoblastoma				
Overall rate	6/50 (12%)	6/50 (12%)	11/50 (22%)	4/50 (8%)
Adjusted rate	15.4%	14.4%	26.1%	9.1%
Terminal rate	2/24 (8%)	3/26 (12%)	5/27 (19%)	3/33 (9%)
First incidence (days)	519	627	456	715
Poly-3 test	P = 0.276N	P = 0.574N	P = 0.181	P = 0.296N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	26/50 (52%)	26/50 (52%)	32/50 (64%)	25/50 (50%)
Adjusted rate	59.2%	56.9%	68.7%	54.1%
Terminal rate	11/24 (46%)	12/26 (46%)	16/27 (59%)	15/33 (46%)
First incidence (days)	455	580	456	534
Poly-3 test	P = 0.395N	P = 0.498N	P = 0.230	P = 0.389N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	45/50 (90%)	47/50 (94%)	48/50 (96%)	42/50 (84%)
Adjusted rate	96.2%	94.8%	98.4%	88.0%
Terminal rate	24/24 (100%)	25/26 (96%)	27/27 (100%)	28/33 (85%)
First incidence (days)	455	407	456	465
Poly-3 test	P = 0.050N	P = 0.573N	P = 0.468	P = 0.114N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	11/50 (22%)	16/50 (32%)	8/50 (16%)
Adjusted rate	16.3%	25.8%	36.1%	18.2%
Terminal rate	6/24 (25%)	5/26 (19%)	8/27 (30%)	6/33 (18%)
First incidence (days)	730 (T)	627	470	709
Poly-3 test	P = 0.504N	P = 0.222	P = 0.037	P = 0.526

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	9/50 (18%)	9/50 (18%)	8/50 (16%)	9/50 (18%)
Adjusted rate	23.2%	21.2%	19.4%	20.6%
Terminal rate	5/24 (21%)	5/26 (19%)	6/27 (22%)	8/33 (24%)
First incidence (days)	455	512	628	724
Poly-3 test	P = 0.448N	P = 0.516N	P = 0.443N	P = 0.490N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	14/50 (28%)	19/50 (38%)	21/50 (42%)	15/50 (30%)
Adjusted rate	36.2%	42.8%	47.0%	34.2%
Terminal rate	10/24 (42%)	9/26 (35%)	12/27 (44%)	12/33 (36%)
First incidence (days)	455	512	470	709
Poly-3 test	P = 0.382N	P = 0.343	P = 0.213	P = 0.517N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Sarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.7%	7.3%	0.0%	2.3%
Terminal rate	1/24 (4%)	2/26 (8%)	0/27 (0%)	1/33 (3%)
First incidence (days)	730 (T)	690	–[e]	730 (T)
Poly-3 test	P = 0.360N	P = 0.343	P = 0.481N	P = 0.721N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	2.4%	0.0%	6.8%
Terminal rate	0/24 (0%)	0/26 (0%)	0/27 (0%)	2/33 (6%)
First incidence (days)	–	704	–	534
Poly-3 test	P = 0.054	P = 0.521	–[f]	P = 0.155
Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	2.4%	2.5%	6.8%
Terminal rate	0/24 (0%)	0/26 (0%)	1/27 (4%)	2/33 (6%)
First incidence (days)	–	704	730 (T)	534
Poly-3 test	P = 0.073	P = 0.521	P = 0.519	P = 0.155
All Organs: Hemangiosarcoma				
Overall rate	6/50 (12%)	5/50 (10%)	4/50 (8%)	4/50 (8%)
Adjusted rate	15.5%	11.9%	9.8%	9.1%
Terminal rate	2/24 (8%)	1/26 (4%)	3/27 (11%)	2/33 (6%)
First incidence (days)	402	583	631	709
Poly-3 test	P = 0.244N	P = 0.443N	P = 0.331N	P = 0.292N

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	7/50 (14%)	4/50 (8%)	5/50 (10%)
Adjusted rate	18.1%	16.7%	9.8%	11.4%
Terminal rate	3/24 (13%)	3/26 (12%)	3/27 (11%)	3/33 (9%)
First incidence (days)	402	583	631	709
Poly-3 test	P = 0.204N	P = 0.551N	P = 0.224N	P = 0.292N
All Organs: Malignant Lymphoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	7.3%	4.9%	0.0%
Terminal rate	0/24 (0%)	2/26 (8%)	2/27 (7%)	0/33 (0%)
First incidence (days)	–	621	730 (T)	–
Poly-3 test	P = 0.322N	P = 0.139	P = 0.259	–
All Organs: Benign Tumors				
Overall rate	36/50 (72%)	44/50 (88%)	44/50 (88%)	43/50 (86%)
Adjusted rate	82.1%	89.7%	91.8%	90.3%
Terminal rate	22/24 (92%)	22/26 (85%)	25/27 (93%)	30/33 (91%)
First incidence (days)	460	407	470	465
Poly-3 test	P = 0.178	P = 0.201	P = 0.112	P = 0.177
All Organs: Malignant Tumors				
Overall rate	36/50 (72%)	40/50 (80%)	38/50 (76%)	32/50 (64%)
Adjusted rate	77.3%	83.6%	81.1%	68.4%
Terminal rate	16/24 (67%)	19/26 (73%)	21/27 (78%)	20/33 (61%)
First incidence (days)	402	512	456	534
Poly-3 test	P = 0.105N	P = 0.298	P = 0.419	P = 0.226N
All Organs: Benign or Malignant Tumors				
Overall rate	48/50 (96%)	50/50 (100%)	48/50 (96%)	46/50 (92%)
Adjusted rate	99.1%	100%	98.4%	96.4%
Terminal rate	24/24 (100%)	26/26 (100%)	27/27 (100%)	32/33 (97%)
First incidence (days)	402	407	456	465
Poly-3 test	P = 0.107N	P = 0.946	P = 0.860N	P = 0.403N

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, liver, and lung; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal kill.
[d]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.
[f]Value of statistic cannot be computed.

Table C-3. Historical Incidence of Alveolar/bronchiolar Neoplasms in Untreated Male B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
β-Picoline (November 2004)	6/50	9/50	14/50
Sodium dichromate dihydrate (September 2002)	15/50	3/50	16/50
Total (%)	21/100 (21.0%)	12/100 (12.0%)	30/100 (30.0%)
Mean ± standard deviation	21.0% ± 12.7%	12.0% ± 8.5%	30.0% ± 2.8%
Range	12%–30%	6%–18%	28%–32%
Overall Historical Incidence: All Routes			
Total (%)	172/1,150 (15.0%)	144/1,150 (12.5%)	301/1,150 (26.2%)
Mean ± standard deviation	15.0% ± 6.9%	12.5% ± 7.1%	26.2% ± 6.3%
Range	2%–30%	4%–24%	14%–40%

[a]Data as of May 4, 2011.

Table C-4. Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	4	8	7
Natural deaths	20	20	15	10
Survivors				
Terminal kill	24	26	27	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(36)	(33)	(39)	(42)
Degeneration, hyaline	–	1 (3%)	–	–
Inflammation, chronic active	–	–	1 (3%)	–
Intestine large, cecum	(35)	(34)	(36)	(40)
Intestine large, colon	(40)	(40)	(41)	(40)
Intestine large, rectum	(39)	(38)	(42)	(41)
Intestine small, duodenum	(36)	(31)	(35)	(40)
Intestine small, ileum	(36)	(35)	(39)	(40)
Intestine small, jejunum	(35)	(32)	(37)	(40)
Diverticulum	1 (3%)	–	–	–
Peyer’s patch, hyperplasia, lymphoid	–	1 (3%)	–	–
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Basophilic focus	10 (20%)	12 (24%)	15 (30%)	7 (14%)
Clear cell focus	15 (30%)	19 (38%)	16 (32%)	9 (18%)
Congestion	–	–	–	1 (2%)
Cyst	–	–	–	1 (2%)
Eosinophilic focus	18 (36%)	16 (32%)	19 (38%)	10 (20%)
Fatty change	–	1 (2%)	–	–
Hematopoietic cell proliferation	1 (2%)	–	–	–
Inflammation, chronic	–	–	2 (4%)	1 (2%)
Inflammation, chronic active	–	–	–	2 (4%)
Mineralization	–	–	1 (2%)	–
Mixed cell focus	5 (10%)	4 (8%)	5 (10%)	4 (8%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Necrosis	5 (10%)	6 (12%)	6 (12%)	4 (8%)
Mesentery	(7)	(1)	(1)	(1)
Inflammation, chronic active	1 (14%)	–	–	–
Fat, necrosis	5 (71%)	–	1 (100%)	1 (100%)
Pancreas	(49)	(48)	(48)	(49)
Atrophy	1 (2%)	–	–	–
Necrosis	–	–	–	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(47)	(47)	(47)	(47)
Hyperplasia, squamous	2 (4%)	4 (9%)	3 (6%)	1 (2%)
Ulcer	5 (11%)	3 (6%)	4 (9%)	1 (2%)
Stomach, glandular	(42)	(45)	(43)	(45)
Mineralization	–	2 (4%)	–	–
Necrosis	–	–	1 (2%)	–
Ulcer	1 (2%)	2 (4%)	–	–
Tooth	(35)	(41)	(42)	(41)
Dysplasia	35 (100%)	41 (100%)	42 (100%)	41 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	12 (24%)	17 (34%)	7 (14%)	4 (8%)
Inflammation, suppurative	2 (4%)	–	–	–
Mineralization	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Necrosis	–	1 (2%)	1 (2%)	–
Thrombosis	2 (4%)	2 (4%)	1 (2%)	–
Artery, inflammation, chronic active	–	–	1 (2%)	2 (4%)
Endocrine System				
Adrenal cortex	(49)	(48)	(50)	(48)
Atrophy	–	1 (2%)	–	–
Hyperplasia	10 (20%)	8 (17%)	9 (18%)	5 (10%)
Hypertrophy	12 (24%)	14 (29%)	14 (28%)	17 (35%)
Subcapsular, hyperplasia	–	1 (2%)	–	–
Adrenal medulla	(49)	(47)	(50)	(48)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	–
Islets, pancreatic	(49)	(48)	(48)	(49)
Hyperplasia	–	1 (2%)	2 (4%)	–
Parathyroid gland	(37)	(39)	(44)	(31)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Pituitary gland	(47)	(49)	(49)	(49)
Thrombosis	1 (2%)	–	–	–
Pars distalis, hyperplasia	1 (2%)	–	2 (4%)	–
Thyroid gland	(49)	(50)	(49)	(49)
General Body System				
None	–	–	–	–
Genital System				
Coagulating gland	(2)	(0)	(0)	(0)
Inflammation, suppurative	1 (50%)	–	–	–
Epididymis	(50)	(50)	(50)	(49)
Granuloma sperm	–	1 (2%)	–	2 (4%)
Mineralization	–	–	–	1 (2%)
Preputial gland	(50)	(50)	(50)	(50)
Cyst	–	–	–	1 (2%)
Inflammation, chronic active	3 (6%)	3 (6%)	4 (8%)	1 (2%)
Prostate	(50)	(50)	(48)	(50)
Hyperplasia	–	1 (2%)	–	–
Inflammation, suppurative	4 (8%)	–	2 (4%)	1 (2%)
Inflammation, chronic active	–	–	1 (2%)	–
Seminal vesicle	(47)	(48)	(49)	(47)
Amyloid deposition	–	–	2 (4%)	1 (2%)
Inflammation, suppurative	1 (2%)	–	–	–
Inflammation, chronic active	–	1 (2%)	3 (6%)	2 (4%)
Testes	(50)	(50)	(50)	(50)
Atrophy	1 (2%)	2 (4%)	2 (4%)	–
Mineralization	–	–	1 (2%)	1 (2%)
Interstitial cell, hyperplasia	–	1 (2%)	–	–
Hematopoietic System				
Bone marrow	(50)	(50)	(48)	(50)
Angiectasis	–	–	1 (2%)	–
Lymph node	(2)	(1)	(0)	(2)
Renal, hyperplasia, lymphoid	1 (50%)	–	–	–
Lymph node, mandibular	(50)	(49)	(49)	(50)
Infiltration cellular, plasma cell	–	–	1 (2%)	–
Lymph node, mesenteric	(45)	(45)	(43)	(45)
Hyperplasia, lymphoid	–	–	–	1 (2%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Inflammation, chronic	–	–	1 (2%)	–
Spleen	(46)	(48)	(47)	(46)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	–	–
Hyperplasia, lymphoid	2 (4%)	–	–	–
Thymus	(41)	(42)	(48)	(47)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	–	–	–
Inflammation, chronic active	2 (4%)	1 (2%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(0)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	–	1 (2%)	–	–
Glial cell, pigmentation	1 (2%)	–	–	–
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)	1 (2%)	–	–
Inflammation, suppurative	–	1 (2%)	–	–
Inflammation, granulomatous	1 (2%)	1 (2%)	–	–
Thrombosis	–	–	1 (2%)	2 (4%)
Alveolar epithelium, hyperplasia	4 (8%)	6 (12%)	6 (12%)	7 (14%)
Alveolus, infiltration cellular, histiocyte	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Bronchiole, hyperplasia	1 (2%)	–	1 (2%)	–
Nose	(50)	(50)	(50)	(50)
Inflammation, suppurative	4 (8%)	9 (18%)	9 (18%)	6 (12%)
Olfactory epithelium, atrophy	3 (6%)	4 (8%)	8 (16%)	7 (14%)
Olfactory epithelium, metaplasia, respiratory	8 (16%)	12 (24%)	30 (60%)	41 (82%)
Respiratory epithelium, hyperplasia	18 (36%)	23 (46%)	23 (46%)	17 (34%)
Pleura	(0)	(1)	(0)	(1)
Trachea	(47)	(49)	(45)	(49)
Special Senses System				
Eye	(36)	(39)	(43)	(40)
Degeneration	1 (3%)	1 (3%)	1 (2%)	1 (3%)
Inflammation, suppurative	–	–	–	1 (3%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Cornea, inflammation, chronic active	–	–	1 (2%)	–
Retina, hemorrhage, chronic	1 (3%)	–	1 (2%)	–
Harderian gland	(46)	(47)	(46)	(47)
Hyperplasia	1 (2%)	1 (2%)	–	4 (9%)
Inflammation, chronic	–	–	1 (2%)	–
Urinary System				
Kidney	(50)	(50)	(50)	(49)
Amyloid deposition	–	–	1 (2%)	–
Cyst	1 (2%)	–	–	–
Hydronephrosis	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Infarct	–	1 (2%)	1 (2%)	–
Inflammation, suppurative	2 (4%)	–	–	1 (2%)
Metaplasia, osseous	4 (8%)	4 (8%)	1 (2%)	4 (8%)
Mineralization	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Nephropathy	43 (86%)	49 (98%)	49 (98%)	49 (100%)
Thrombosis	1 (2%)	–	–	–
Artery, inflammation, chronic active	–	1 (2%)	1 (2%)	–
Papilla, necrosis	2 (4%)	–	1 (2%)	2 (4%)
Renal tubule, hyperplasia	–	–	1 (2%)	–
Renal tubule, hypertrophy	–	1 (2%)	–	–
Renal tubule, pigmentation	1 (2%)	–	–	1 (2%)
Urethra	(3)	(3)	(5)	(3)
Inflammation, suppurative	1 (33%)	3 (100%)	3 (60%)	1 (33%)
Urinary bladder	(45)	(45)	(45)	(46)
Hemorrhage	1 (2%)	1 (2%)	–	–
Inflammation, suppurative	1 (2%)	–	–	–
Inflammation, chronic	–	–	1 (2%)	–

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix D. Summary of Lesions in Female Mice in the Two-year Drinking Water Study of β-Picoline

Tables

Table D-1. Summary of the Incidence of Neoplasms in Female Mice in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	3	1	4
Natural deaths	8	15	14	13
Survivors				
Died last week of study	–	–	1	–
Terminal kill	38	32	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(49)	(50)
Gallbladder	(43)	(36)	(36)	(35)
Hepatoblastoma, metastatic, liver	–	1 (3%)	–	–
Intestine large, cecum	(44)	(36)	(38)	(39)
Carcinoma	–	–	1 (3%)	–
Intestine large, colon	(44)	(35)	(40)	(38)
Intestine large, rectum	(43)	(35)	(37)	(38)
Intestine small, duodenum	(42)	(35)	(36)	(37)
Intestine small, ileum	(44)	(36)	(37)	(37)
Intestine small, jejunum	(43)	(35)	(37)	(38)
Carcinoma	–	–	–	2 (5%)
Liver	(49)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	5 (10%)	3 (6%)	2 (4%)
Hepatoblastoma	1 (2%)	3 (6%)	3 (6%)	3 (6%)
Hepatoblastoma, multiple	–	–	1 (2%)	1 (2%)
Hepatocellular adenoma	11 (22%)	3 (6%)	10 (20%)	9 (18%)
Hepatocellular adenoma, multiple	27 (55%)	43 (86%)	36 (72%)	30 (60%)
Hepatocellular carcinoma	6 (12%)	13 (26%)	16 (32%)	18 (36%)
Hepatocellular carcinoma, multiple	5 (10%)	7 (14%)	10 (20%)	5 (10%)
Mast cell tumor malignant	–	1 (2%)	–	–
Osteosarcoma, metastatic, bone	–	–	–	1 (2%)
Rhabdomyosarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Mesentery	(8)	(16)	(16)	(9)
Lipoma	–	–	1 (6%)	–

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Rhabdomyosarcoma, metastatic, uncertain primary site	–	–	1 (6%)	–
Sarcoma	–	–	–	1 (11%)
Pancreas	(48)	(42)	(46)	(45)
Rhabdomyosarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Sarcoma, metastatic, mesentery	–	–	–	1 (2%)
Salivary glands	(50)	(47)	(47)	(50)
Stomach, forestomach	(47)	(43)	(45)	(47)
Squamous cell carcinoma	–	1 (2%)	–	–
Squamous cell papilloma	–	1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(45)	(39)	(41)	(41)
Tongue	(0)	(0)	(0)	(1)
Squamous cell carcinoma	–	–	–	1 (100%)
Tooth	(0)	(1)	(2)	(9)
Gingiva, fibroma, osseous	–	1 (100%)	–	–
Cardiovascular System				
Blood vessel	(2)	(3)	(1)	(0)
Heart	(50)	(50)	(49)	(50)
Hemangiosarcoma	–	–	–	1 (2%)
Pericardium, alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Endocrine System				
Adrenal cortex	(49)	(45)	(46)	(50)
Adenoma	–	–	–	1 (2%)
Sarcoma, metastatic, mesentery	–	–	–	1 (2%)
Adrenal medulla	(49)	(45)	(46)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)	–	–
Pheochromocytoma complex	–	–	1 (2%)	–
Pheochromocytoma malignant	–	2 (4%)	–	–
Islets, pancreatic	(48)	(41)	(45)	(45)
Adenoma	–	1 (2%)	–	1 (2%)
Parathyroid gland	(38)	(34)	(40)	(41)
Pituitary gland	(50)	(46)	(47)	(46)
Pars distalis, adenoma	4 (8%)	5 (11%)	2 (4%)	3 (7%)
Thyroid gland	(48)	(45)	(45)	(49)
Follicular cell, adenoma	–	–	–	1 (2%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(50)	(48)	(50)
Ovary	(49)	(48)	(48)	(47)
Cystadenoma	3 (6%)	4 (8%)	–	6 (13%)
Hemangioma	–	–	–	1 (2%)
Uterus	(49)	(47)	(49)	(49)
Hemangiosarcoma	–	–	–	1 (2%)
Polyp stromal	2 (4%)	–	–	–
Cervix, sarcoma	–	–	1 (2%)	–
Endometrium, adenoma	1 (2%)	–	–	–
Hematopoietic System				
Bone marrow	(49)	(46)	(48)	(49)
Hemangiosarcoma	1 (2%)	–	–	–
Mast cell tumor malignant	–	1 (2%)	–	–
Lymph node	(6)	(8)	(6)	(8)
Bronchial, alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (13%)
Iliac, mast cell tumor malignant	–	1 (13%)	–	–
Iliac, sarcoma, metastatic, mesentery	–	–	–	1 (13%)
Lumbar, hemangiosarcoma	–	1 (13%)	–	–
Mediastinal, sarcoma, metastatic, mesentery	–	–	–	1 (13%)
Renal, hemangiosarcoma	–	1 (13%)	–	–
Lymph node, mandibular	(48)	(42)	(47)	(47)
Sarcoma, metastatic, mesentery	–	–	–	1 (2%)
Lymph node, mesenteric	(46)	(38)	(45)	(46)
Sarcoma, metastatic, mesentery	–	–	–	1 (2%)
Spleen	(46)	(42)	(45)	(46)
Fibrosarcoma	–	–	–	1 (2%)
Hemangiosarcoma	1 (2%)	2 (5%)	–	–
Mast cell tumor malignant	–	1 (2%)	–	–
Thymus	(45)	(44)	(48)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	1 (2%)	–	2 (4%)
Hemangiosarcoma	–	–	1 (2%)	–
Rhabdomyosarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Sarcoma, metastatic, mesentery	–	–	–	1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(49)	(48)
Carcinoma	–	1 (2%)	–	–
Skin	(50)	(50)	(50)	(50)
Mast cell tumor benign	1 (2%)	–	–	–
Subcutaneous tissue, fibrous histiocytoma	–	1 (2%)	–	–
Subcutaneous tissue, hemangiosarcoma	2 (4%)	1 (2%)	–	–
Subcutaneous tissue, rhabdomyosarcoma, metastatic, uncertain primary site	–	–	1 (2%)	–
Subcutaneous tissue, sarcoma	2 (4%)	2 (4%)	4 (8%)	4 (8%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	2 (4%)	–	–	–
Osteosarcoma, multiple	–	–	–	1 (2%)
Skeletal muscle	(1)	(0)	(0)	(1)
Sarcoma, metastatic, mesentery	–	–	–	1 (100%)
Nervous System				
Brain	(50)	(47)	(47)	(50)
Peripheral nerve	(1)	(0)	(0)	(1)
Spinal cord	(1)	(0)	(0)	(1)
Respiratory System				
Lung	(50)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	5 (10%)	4 (8%)	10 (20%)
Alveolar/bronchiolar adenoma, multiple	–	1 (2%)	–	1 (2%)
Alveolar/bronchiolar carcinoma	7 (14%)	6 (12%)	8 (16%)	9 (18%)
Alveolar/bronchiolar carcinoma, multiple	–	2 (4%)	2 (4%)	4 (8%)
Carcinoma, metastatic, harderian gland	1 (2%)	–	3 (6%)	–
Hepatoblastoma, metastatic, liver	–	–	–	1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	5 (10%)	8 (16%)	4 (8%)
Mast cell tumor malignant	–	1 (2%)	–	–
Osteosarcoma, metastatic, bone	–	–	–	1 (2%)
Sarcoma, metastatic, mesentery	–	–	–	1 (2%)
Sarcoma, metastatic, skin	1 (2%)	–	1 (2%)	–
Nose	(49)	(44)	(49)	(47)
Pleura	(0)	(0)	(0)	(1)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (100%)
Trachea	(47)	(41)	(45)	(44)
Special Senses System				
Eye	(43)	(35)	(36)	(39)
Carcinoma, metastatic, harderian gland	1 (2%)	–	–	–
Harderian gland	(45)	(41)	(43)	(47)
Adenoma	2 (4%)	–	2 (5%)	2 (4%)
Carcinoma	2 (4%)	1 (2%)	4 (9%)	4 (9%)
Bilateral, carcinoma	–	1 (2%)	–	–
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(49)	(47)	(48)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung	–	–	–	1 (2%)
Mast cell tumor malignant	–	1 (2%)	–	–
Osteosarcoma, metastatic, bone	–	–	–	1 (2%)
Renal tubule, adenoma	–	–	–	1 (2%)
Urinary bladder	(44)	(37)	(39)	(41)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	–	1 (2%)	1 (2%)
Lymphoma malignant	14 (28%)	15 (30%)	10 (20%)	13 (26%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	43	49	50	47
Total primary neoplasms	103	136	122	139
Total animals with benign neoplasms	40	48	47	44
Total benign neoplasms	57	65	56	67
Total animals with malignant neoplasms	29	42	41	41
Total malignant neoplasms	46	71	66	72
Total animals with metastatic neoplasms	3	7	12	7
Total metastatic neoplasms	4	7	17	23
Total animals with malignant neoplasms of uncertainprimary site	–	–	1	–

[a]Number of animals examined microscopically at the site and the number of animals with neoplasm.
[b]Number of animals with any tissue examined microscopically.
[c]Primary neoplasms: all neoplasms except metastatic neoplasms.

Table D-2. Statistical Analysis of Primary Neoplasms in Female Mice in the Two-year Drinking Water Study of β-Picoline

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate[a]	1/49 (2%)	3/45 (7%)	1/46 (2%)	0/50 (0%)
Adjusted rate[b]	2.2%	7.2%	2.3%	0.0%
Terminal rate[c]	1/38 (3%)	2/32 (6%)	0/35 (0%)	0/33 (0%)
First incidence (days)	729 (T)	553	704	–[e]
Poly-3 test[d]	P = 0.209N	P = 0.274	P = 0.745	P = 0.508N
Harderian Gland: Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	4/50 (8%)	4/50 (8%)
Adjusted rate	4.3%	4.5%	8.8%	9.0%
Terminal rate	1/38 (3%)	2/32 (6%)	4/35 (11%)	3/33 (9%)
First incidence (days)	668	729 (T)	729 (T)	533
Poly-3 test	P = 0.201	P = 0.680	P = 0.329	P = 0.322
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	6/50 (12%)	6/50 (12%)
Adjusted rate	8.7%	4.5%	13.1%	13.3%
Terminal rate	3/38 (8%)	2/32 (6%)	4/35 (11%)	4/33 (12%)
First incidence (days)	668	729 (T)	647	533
Poly-3 test	P = 0.174	P = 0.356N	P = 0.364	P = 0.354
Liver: Hemangiosarcoma				
Overall rate	2/49 (4%)	5/50 (10%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.4%	11.3%	6.6%	4.5%
Terminal rate	2/38 (5%)	4/32 (13%)	3/35 (9%)	1/33 (3%)
First incidence (days)	729 (T)	724	729 (T)	648
Poly-3 test	P = 0.441N	P = 0.203	P = 0.496	P = 0.682
Liver: Hepatocellular Adenoma				
Overall rate	38/49 (78%)	46/50 (92%)	46/50 (92%)	39/50 (78%)
Adjusted rate	80.4%	94.9%	93.1%	81.5%
Terminal rate	31/38 (82%)	31/32 (97%)	35/35 (100%)	26/33 (79%)
First incidence (days)	598	494	309	509
Poly-3 test	P = 0.403N	P = 0.025	P = 0.052	P = 0.551

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Liver: Hepatocellular Carcinoma				
Overall rate	11/49 (22%)	20/50 (40%)	26/50 (52%)	23/50 (46%)
Adjusted rate	23.6%	43.9%	55.3%	50.9%
Terminal rate	7/38 (18%)	14/32 (44%)	21/35 (60%)	18/33 (55%)
First incidence (days)	639	533	549	586
Poly-3 test	P = 0.006	P = 0.031	P < 0.001	P = 0.005
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	40/49 (82%)	47/50 (94%)	47/50 (94%)	42/50 (84%)
Adjusted rate	84%	96.9%	95%	86.2%
Terminal rate	32/38 (84%)	31/32 (97%)	35/35 (100%)	27/33 (82%)
First incidence (days)	598	494	309	509
Poly-3 test	P = 0.496N	P = 0.027	P = 0.066	P = 0.492
Liver: Hepatoblastoma				
Overall rate	1/49 (2%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	2.2%	6.7%	8.8%	9.1%
Terminal rate	0/38 (0%)	1/32 (3%)	4/35 (11%)	3/33 (9%)
First incidence (days)	674	662	729 (T)	721
Poly-3 test	P = 0.140	P = 0.295	P = 0.174	P = 0.166
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	12/49 (24%)	21/50 (42%)	28/50 (56%)	24/50 (48%)
Adjusted rate	25.7%	45.8%	59.5%	53.1%
Terminal rate	7/38 (18%)	14/32 (44%)	23/35 (66%)	19/33 (58%)
First incidence (days)	639	533	549	586
Poly-3 test	P = 0.005	P = 0.033	P < 0.001	P = 0.005
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	40/49 (82%)	47/50 (94%)	47/50 (94%)	42/50 (84%)
Adjusted rate	84%	96.9%	95.0%	86.2%
Terminal rate	32/38 (84%)	31/32 (97%)	35/35 (100%)	27/33 (82%)
First incidence (days)	598	494	309	509
Poly-3 test	P = 0.496N	P = 0.027	P = 0.066	P = 0.492
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	5/50 (10%)	6/50 (12%)	4/49 (8%)	11/50 (22%)
Adjusted rate	10.9%	13.5%	8.9%	24.5%
Terminal rate	5/38 (13%)	5/32 (16%)	3/35 (9%)	8/33 (24%)
First incidence (days)	729 (T)	700	669	533
Poly-3 test	P = 0.046	P = 0.477	P = 0.511N	P = 0.075

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	7/50 (14%)	8/50 (16%)	10/49 (20%)	13/50 (26%)
Adjusted rate	15.2%	17.9%	21.6%	28.5%
Terminal rate	6/38 (16%)	6/32 (19%)	7/35 (20%)	8/33 (24%)
First incidence (days)	669	700	522	509
Poly-3 test	P = 0.061	P = 0.471	P = 0.297	P = 0.096
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	11/50 (22%)	13/50 (26%)	13/49 (27%)	21/50 (42%)
Adjusted rate	23.8%	29.1%	28.1%	45.4%
Terminal rate	10/38 (26%)	11/32 (34%)	10/35 (29%)	14/33 (42%)
First incidence (days)	669	700	522	509
Poly-3 test	P = 0.015	P = 0.368	P = 0.407	P = 0.022
Ovary: Cystadenoma				
Overall rate	3/49 (6%)	4/48 (8%)	0/48 (0%)	6/47 (13%)
Adjusted rate	6.5%	9.1%	0.0%	14.3%
Terminal rate	3/38 (8%)	3/32 (9%)	0/35 (0%)	6/33 (18%)
First incidence (days)	729 (T)	724	–	729 (T)
Poly-3 test	P = 0.177	P = 0.477	P = 0.125N	P = 0.201
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/50 (8%)	5/46 (11%)	2/47 (4%)	3/46 (7%)
Adjusted rate	8.7%	11.9%	4.6%	7.4%
Terminal rate	4/38 (11%)	4/31 (13%)	1/35 (3%)	3/30 (10%)
First incidence (days)	729 (T)	494	712	729 (T)
Poly-3 test	P = 0.373N	P = 0.443	P = 0.364N	P = 0.570N
Skin: Sarcoma				
Overall rate	2/50 (4%)	2/50 (4%)	4/50 (8%)	4/50 (8%)
Adjusted rate	4.3%	4.5%	8.7%	9.0%
Terminal rate	0/38 (0%)	1/32 (3%)	1/35 (3%)	2/33 (6%)
First incidence (days)	698	700	620	612
Poly-3 test	P = 0.200	P = 0.681	P = 0.337	P = 0.321
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Sarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	4.3%	6.7%	8.7%	9%
Terminal rate	0/38 (0%)	2/32 (6%)	1/35 (3%)	2/33 (6%)
First incidence (days)	698	700	620	612
Poly-3 test	P = 0.251	P = 0.484	P = 0.337	P = 0.321

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Spleen: Hemangiosarcoma				
Overall rate	1/46 (2%)	2/42 (5%)	0/45 (0%)	0/46 (0%)
Adjusted rate	2.3%	5.0%	0.0%	0.0%
Terminal rate	1/38 (3%)	1/32 (3%)	0/35 (0%)	0/33 (0%)
First incidence (days)	729 (T)	588	–	–
Poly-3 test	P = 0.194N	P = 0.463	P = 0.511N	P = 0.512N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	6/50 (12%)	4/50 (8%)	4/50 (8%)
Adjusted rate	6.5%	13.4%	8.8%	9.0%
Terminal rate	3/38 (8%)	4/32 (13%)	4/35 (11%)	2/33 (6%)
First incidence (days)	729 (T)	588	729 (T)	641
Poly-3 test	P = 0.533	P = 0.230	P = 0.493	P = 0.485
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	6/50 (12%)	4/50 (8%)	5/50 (10%)
Adjusted rate	6.5%	13.4%	8.8%	11.1%
Terminal rate	3/38 (8%)	4/32 (13%)	4/35 (11%)	2/33 (6%)
First incidence (days)	729 (T)	588	729 (T)	573
Poly-3 test	P = 0.392	P = 0.230	P = 0.493	P = 0.347
All Organs: Malignant Lymphoma				
Overall rate	14/50 (28%)	15/50 (30%)	10/50 (20%)	13/50 (26%)
Adjusted rate	29.7%	33.0%	21.9%	28.9%
Terminal rate	10/38 (26%)	10/32 (31%)	7/35 (20%)	10/33 (30%)
First incidence (days)	598	382	686	612
Poly-3 test	P = 0.413N	P = 0.457	P = 0.265N	P = 0.554N
All Organs: Benign Tumors				
Overall rate	40/50 (80%)	48/50 (96%)	47/50 (94%)	44/50 (88%)
Adjusted rate	84.4%	99.0%	94.5%	89.2%
Terminal rate	33/38 (87%)	32/32 (100%)	35/35 (100%)	28/33 (85%)
First incidence (days)	598	494	309	509
Poly-3 test	P = 0.512	P = 0.007	P = 0.084	P = 0.338
All Organs: Malignant Tumors				
Overall rate	29/50 (58%)	42/50 (84%)	41/50 (82%)	41/50 (82%)
Adjusted rate	60.7%	87.0%	84.2%	84.0%
Terminal rate	21/38 (55%)	27/32 (84%)	29/35 (83%)	26/33 (79%)
First incidence (days)	598	382	522	509
Poly-3 test	P = 0.015	P = 0.002	P = 0.007	P = 0.008

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
All Organs: Benign or Malignant Tumors				
Overall rate	43/50 (86%)	49/50 (98%)	50/50 (100%)	47/50 (94%)
Adjusted rate	89.7%	99.3%	100.0%	95.3%
Terminal rate	34/38 (90%)	32/32 (100%)	35/35 (100%)	31/33 (94%)
First incidence (days)	598	382	309	509
Poly-3 test	P = 0.237	P = 0.038	P = 0.024	P = 0.245

(T) Terminal kill.
[a]Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, ovary, pituitary gland, and spleen; for other tissues, denominator is number of animals necropsied.
[b]Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality.
[c]Observed incidence at terminal kill.
[d]Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal kill. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[e]Not applicable; no neoplasms in animal group.

Table D-3. Historical Incidence of Neoplasms of the Liver in Untreated Female B6C3F1/N Mice[a]

Study (Study Start)	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma	Hepatocellular Carcinoma or Hepatoblastoma
Historical Incidence: Drinking Water Studies				
β-Picoline (November 2004)	38/49	11/49	1/49	12/49
Sodium dichromate dihydrate (September 2002)	14/49	8/49	0/49	8/49
Total (%)	52/98 (53.1%)	19/98 (19.4%)	1/98 (1.0%)	20/98 (20.4%)
Mean ± standard deviation	53.1% ± 34.6%	19.4% ± 4.3%	1.0% ± 1.4%	20.4% ± 5.8%
Range	29%–78%	16%–22%	0%–2%	16%–24%
Overall Historical Incidence: All Routes				
Total (%)	380/1,195 (31.8%)	144/1,195 (12.1%)	4/1,195 (0.3%)	148/1,195 (12.4%)
Mean ± standard deviation	31.8% ± 21.4%	12.1% ± 10.8%	0.3% ± 0.8%	12.4% ± 11.2%
Range	2%–78%	0%–46%	0%–2%	0%–46%

[a]Data as of May 4, 2011.

Table D-4. Historical Incidence of Alveolar/Bronchiolar Neoplasms in Untreated Female B6C3F1/N Mice[a]

Study (Study Start)	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
β-Picoline (November 2004)	5/50	7/50	11/50
Sodium dichromate dihydrate (September 2002)	1/50	2/50	2/50
Total (%)	6/100 (6.0%)	9/100 (9.0%)	13/100 (13.0%)
Mean ± standard deviation	6.0% ± 5.7%	9.0% ± 7.1%	13.0% ± 12.7%
Range	2%–10%	4%–14%	4%–22%
Overall Historical Incidence: All Routes			
Total (%)	60/1,196 (5.0%)	44/1,196 (3.7%)	100/1,196 (8.4%)
Mean ± standard deviation	5.0% ± 3.6%	3.7% ± 3.3%	8.4% ± 4.3%
Range	0%–12%	0%–14%	2%–22%

[a]Data as of May 4, 2011.

Table D-5. Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the Two-year Drinking Water Study of β-Picoline[a]

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	3	1	4
Natural deaths	8	15	14	13
Survivors				
Died last week of study	–	–	1	–
Terminal kill	38	32	34	33
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(49)	(49)	(50)
Gallbladder	(43)	(36)	(36)	(35)
Degeneration, hyaline	–	–	1 (3%)	1 (3%)
Intestine large, cecum	(44)	(36)	(38)	(39)
Intestine large, colon	(44)	(35)	(40)	(38)
Intestine large, rectum	(43)	(35)	(37)	(38)
Intestine small, duodenum	(42)	(35)	(36)	(37)
Lymphangiectasis	–	–	1 (3%)	–
Intestine small, ileum	(44)	(36)	(37)	(37)
Inflammation, acute	–	–	1 (3%)	–
Intestine small, jejunum	(43)	(35)	(37)	(38)
Inflammation, granulomatous	–	1 (3%)	–	–
Inflammation, acute	–	–	1 (3%)	–
Liver	(49)	(50)	(50)	(50)
Angiectasis	3 (6%)	3 (6%)	6 (12%)	5 (10%)
Basophilic focus	4 (8%)	2 (4%)	1 (2%)	6 (12%)
Clear cell focus	3 (6%)	2 (4%)	6 (12%)	–
Congestion	–	–	–	1 (2%)
Eosinophilic focus	11 (22%)	10 (20%)	14 (28%)	15 (30%)
Fatty change	–	4 (8%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	–	1 (2%)	–	–
Mixed cell focus	–	–	–	2 (4%)
Necrosis	4 (8%)	2 (4%)	3 (6%)	2 (4%)
Tension lipidosis	1 (2%)	1 (2%)	–	2 (4%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Thrombosis	–	1 (2%)	–	–
Mesentery	(8)	(16)	(16)	(9)
Fat, hemorrhage	–	1 (6%)	–	–
Fat, necrosis	7 (88%)	14 (88%)	13 (81%)	8 (89%)
Pancreas	(48)	(42)	(46)	(45)
Atrophy	2 (4%)	–	2 (4%)	1 (2%)
Thrombosis	–	1 (2%)	–	–
Duct, cyst	1 (2%)	1 (2%)	–	–
Salivary glands	(50)	(47)	(47)	(50)
Inflammation, chronic	–	–	–	1 (2%)
Necrosis	1 (2%)	–	–	–
Stomach, forestomach	(47)	(43)	(45)	(47)
Hyperplasia, squamous	–	1 (2%)	3 (7%)	3 (6%)
Ulcer	–	1 (2%)	1 (2%)	–
Stomach, glandular	(45)	(39)	(41)	(41)
Dysplasia	–	–	1 (2%)	–
Hyperplasia	–	1 (3%)	–	–
Mineralization	2 (4%)	2 (5%)	1 (2%)	–
Tongue	(0)	(0)	(0)	(1)
Tooth	(0)	(1)	(2)	(9)
Dysplasia	–	–	2 (100%)	9 (100%)
Cardiovascular System				
Blood vessel	(2)	(3)	(1)	(0)
Mineralization	2 (100%)	3 (100%)	1 (100%)	–
Heart	(50)	(50)	(49)	(50)
Cardiomyopathy	4 (8%)	1 (2%)	7 (14%)	3 (6%)
Inflammation, suppurative	1 (2%)	1 (2%)	–	1 (2%)
Mineralization	1 (2%)	4 (8%)	1 (2%)	–
Necrosis	–	1 (2%)	–	–
Thrombosis	2 (4%)	1 (2%)	–	–
Artery, inflammation, chronic active	–	–	–	1 (2%)
Capillary, hyperplasia	–	1 (2%)	–	–
Endocrine System				
Adrenal cortex	(49)	(45)	(46)	(50)
Hyperplasia	2 (4%)	–	–	1 (2%)
Hypertrophy	1 (2%)	1 (2%)	–	1 (2%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Vacuolization cytoplasmic	–	–	1 (2%)	–
Adrenal medulla	(49)	(45)	(46)	(50)
Hyperplasia	–	1 (2%)	–	–
Islets, pancreatic	(48)	(41)	(45)	(45)
Hyperplasia	–	–	1 (2%)	1 (2%)
Parathyroid gland	(38)	(34)	(40)	(41)
Hyperplasia	–	–	2 (5%)	–
Pituitary gland	(50)	(46)	(47)	(46)
Pars distalis, angiectasis	3 (6%)	1 (2%)	–	1 (2%)
Pars distalis, hyperplasia	8 (16%)	14 (30%)	13 (28%)	7 (15%)
Thyroid gland	(48)	(45)	(45)	(49)
Cyst	–	–	–	2 (4%)
Follicular cell, hyperplasia	1 (2%)	–	–	1 (2%)
Follicular cell, hypertrophy	–	–	1 (2%)	–
General Body System				
None	–	–	–	–
Genital System				
Clitoral gland	(50)	(50)	(48)	(50)
Ovary	(49)	(48)	(48)	(47)
Cyst	5 (10%)	5 (10%)	5 (10%)	4 (9%)
Thrombosis	–	–	2 (4%)	4 (9%)
Uterus	(49)	(47)	(49)	(49)
Angiectasis	–	1 (2%)	–	–
Inflammation, acute	–	–	–	2 (4%)
Necrosis	–	–	–	1 (2%)
Thrombosis	1 (2%)	–	1 (2%)	–
Endometrium, hyperplasia, cystic	33 (67%)	29 (62%)	33 (67%)	37 (76%)
Hematopoietic System				
Bone marrow	(49)	(46)	(48)	(49)
Angiectasis	–	–	–	1 (2%)
Lymph node	(6)	(8)	(6)	(8)
Lumbar, angiectasis	1 (17%)	–	–	–
Lumbar, hemorrhage	–	–	1 (17%)	–
Mediastinal, hyperplasia, lymphoid	1 (17%)	1 (13%)	–	–
Pancreatic, ectasia	1 (17%)	–	–	–
Renal, ectasia	1 (17%)	1 (13%)	1 (17%)	–

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Lymph node, mandibular	(48)	(42)	(47)	(47)
Hyperplasia, lymphoid	–	–	1 (2%)	–
Lymph node, mesenteric	(46)	(38)	(45)	(46)
Angiectasis	–	–	–	1 (2%)
Hemorrhage	–	–	1 (2%)	1 (2%)
Spleen	(46)	(42)	(45)	(46)
Hematopoietic cell proliferation	4 (9%)	3 (7%)	5 (11%)	1 (2%)
Hyperplasia, lymphoid	–	–	–	1 (2%)
Thrombosis	–	1 (2%)	–	–
Thymus	(45)	(44)	(48)	(47)
Amyloid deposition	–	–	1 (2%)	–
Hyperplasia, lymphoid	–	–	1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(50)	(49)	(49)	(48)
Hyperplasia	1 (2%)	–	–	–
Skin	(50)	(50)	(50)	(50)
Hyperplasia, squamous	–	1 (2%)	–	–
Inflammation, acute	–	–	1 (2%)	–
Inflammation, chronic active	–	2 (4%)	–	–
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis	–	–	–	1 (2%)
Skeletal muscle	(1)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(47)	(47)	(50)
Hemorrhage	–	1 (2%)	–	1 (2%)
Necrosis	–	1 (2%)	–	–
Peripheral nerve	(1)	(0)	(0)	(1)
Spinal cord	(1)	(0)	(0)	(1)
Respiratory System				
Lung	(50)	(50)	(49)	(50)
Hemorrhage	–	–	–	1 (2%)
Inflammation, acute	–	–	1 (2%)	–
Inflammation, chronic active	–	1 (2%)	–	–
Mineralization	1 (2%)	3 (6%)	2 (4%)	–
Alveolar epithelium, hyperplasia	2 (4%)	4 (8%)	3 (6%)	8 (16%)

	0 mg/L	312.5 mg/L	625 mg/L	1,250 mg/L
Alveolus, infiltration cellular, histiocyte	2 (4%)	1 (2%)	1 (2%)	–
Bronchiole, hyperplasia	–	–	3 (6%)	1 (2%)
Nose	(49)	(44)	(49)	(47)
Inflammation, suppurative	1 (2%)	–	–	1 (2%)
Olfactory epithelium, atrophy	1 (2%)	2 (5%)	2 (4%)	7 (15%)
Olfactory epithelium, metaplasia, respiratory	2 (4%)	2 (5%)	7 (14%)	14 (30%)
Respiratory epithelium, hyperplasia	7 (14%)	2 (5%)	6 (12%)	13 (28%)
Pleura	(0)	(0)	(0)	(1)
Trachea	(47)	(41)	(45)	(44)
Mineralization	1 (2%)	1 (2%)	–	–
Special Senses System				
Eye	(43)	(35)	(36)	(39)
Cataract	1 (2%)	–	–	–
Degeneration	1 (2%)	–	1 (3%)	1 (3%)
Cornea, inflammation, acute	1 (2%)	1 (3%)	–	–
Cornea, inflammation, chronic active	–	–	–	1 (3%)
Harderian gland	(45)	(41)	(43)	(47)
Hyperplasia	5 (11%)	2 (5%)	1 (2%)	1 (2%)
Inflammation, chronic	–	–	1 (2%)	–
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(49)	(47)	(48)	(49)
Amyloid deposition	1 (2%)	1 (2%)	1 (2%)	–
Hydronephrosis	–	1 (2%)	–	–
Inflammation, suppurative	1 (2%)	–	–	1 (2%)
Metaplasia, osseous	–	1 (2%)	1 (2%)	–
Mineralization	3 (6%)	2 (4%)	1 (2%)	2 (4%)
Nephropathy	29 (59%)	16 (34%)	25 (52%)	30 (61%)
Renal tubule, necrosis	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Urinary bladder	(44)	(37)	(39)	(41)
Inflammation, chronic	–	–	–	1 (2%)

[a]Number of animals examined microscopically at the site and the number of animals with lesion.

Appendix E. Genetic Toxicology

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Tables

E.1. Bacterial Mutagenicity Test Protocol

Testing procedures used in the first two studies, conducted at EG&G Mason Research Institute (Rockville, MD) and BioReliance Corporation (Rockville, MD), followed the protocol reported by Zeiger et al.[61]; in the tests conducted at SITEK Research Laboratories (Rockville, MD), using the same chemical lot 11108CI that was tested in the 3-month and 2-year drinking water studies, a slightly modified procedure was used, and that is described in more detail below. β-Picoline was tested as a coded sample. In the first two studies, β-picoline was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA1535, and TA1537 either in buffer or in 10% or 30% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver) for 20 minutes at 37°C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

The protocol used at SITEK Research Laboratories used only 10% rat liver S9 for exogenous metabolic activation, and employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strains TA98 and TA100. Incubation of bacterial strains with β-picoline and subsequent plating were carried out as described above.

In all studies, each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of β-picoline; the highest concentration tested was limited by toxicity. All trials were repeated at the same or a higher S9 concentration.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose-related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold-increase required for a chemical to be judged positive or weakly positive, although positive calls are typically reserved for increases in mutant colonies that are at least twofold over background.

E.2. Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[62]. At the end of the 3-month drinking water study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were shipped to the genetic toxicity testing laboratory where they were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of five animals per exposure group. In addition, the percentage of polychromatic erythrocytes (PCEs) among the total erythrocyte population in the peripheral blood was scored for each exposure group as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group, plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed group and the control group. In the presence of excess

binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests, because additional test data could not be obtained. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

E.3. Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the in vitro assays have another variable that must be considered in arriving at an overall test result. In vitro assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

E.4. Results

β-Picoline was tested in three independent bacterial gene mutation studies, and negative results were obtained in all studies (Table E-1 and Table E-2). In the first study (with concentrations ranging from 85.4 to 8,540 μg/plate), no increases in the numbers of mutant colonies were seen in *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without 10% S9 derived from induced hamster or rat liver. In the second study, negative results were obtained over a concentration range of 100 to 10,000 μg/plate in *S. typhimurium* strains TA97, TA98, TA100, and TA1535 with and without 10% or 30% S9 derived from induced hamster or rat liver. In the third study, which tested the same chemical lot that was used in the 3-month and 2-year studies, negative results were obtained over a concentration range of 1,000 to 10,000 μg/plate in *S. typhimurium* strains TA98 and TA100 and 100 to 5,000 μg/plate in *E. coli* WP2 *uvrA*/pKM101, with and without 10% rat liver S9.

In vivo, no significant increases in the frequencies of micronucleated NCEs, an indicator of chromosomal damage, were observed in peripheral blood of male or female B6C3F1/N mice exposed to 78 to 1,250 mg β-picoline/L in drinking water for 3 months (Table E-3). No significant alterations in the percentage of circulating PCEs (reticulocytes) were observed, suggesting that β-picoline did not induce bone marrow toxicity over the exposure concentration range tested.

Table E-1. Mutagenicity of β-Picoline in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Without S9	Without S9	With 10% Hamster S9	With 10% Hamster S9	With 10% Rat S9	With 10% Rat S9
Study performed at EG&G Mason Research Institute							
TA100							
	0	118 ± 1	122 ± 11	106 ± 13	131 ± 6	121 ± 9	133 ± 2
	85.4	111 ± 6	132 ± 6	118 ± 6	144 ± 3	128 ± 5	156 ± 4
	284.7	107 ± 14	115 ± 6	112 ± 5	132 ± 7	120 ± 7	123 ± 5
	854	111 ± 12	115 ± 13	113 ± 7	146 ± 11	140 ± 4	119 ± 10
	2,846	127 ± 11	113 ± 7	108 ± 17	133 ± 6	121 ± 2	117 ± 5
	8,540	Toxic	Toxic	Toxic	65 ± 5[b]	71 ± 8[b]	72 ± 19[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		2,008 ± 50	1,578 ± 69	2,101 ± 19	2,930 ± 69	1,959 ± 21	2,561 ± 99
TA98							
	0	18 ± 3	17 ± 1	19 ± 4	20 ± 5	23 ± 1	25 ± 3
	85.4	14 ± 1	15 ± 2	19 ± 4	23 ± 3	19 ± 2	19 ± 3
	284.7	14 ± 2	17 ± 2	24 ± 4	23 ± 3	14 ± 1	23 ± 5
	854	16 ± 1	16 ± 2	22 ± 3	21 ± 1	20 ± 5	15 ± 2
	2,846	13 ± 2	14 ± 2	26 ± 2	20 ± 2	19 ± 2	22 ± 4
	8,540	Toxic	Toxic	9 ± 2[b]	13 ± 1[b]	10 ± 1[b]	15 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,931 ± 5	1,191 ± 14	2,110 ± 100	2,862 ± 38	1,686 ± 68	2,029 ± 62
TA1535							
	0	24 ± 2	15 ± 0	4 ± 1	10 ± 2	10 ± 2	9 ± 3
	85.4	19 ± 1	11 ± 1	8 ± 1	9 ± 2	8 ± 2	10 ± 2
	284.7	21 ± 1	15 ± 1	10 ± 4	8 ± 2	8 ± 3	10 ± 1
	854	22 ± 2	12 ± 1	8 ± 1	6 ± 1	7 ± 2	11 ± 1
	2,846	19 ± 3	16 ± 2	10 ± 1	7 ± 1	8 ± 1	10 ± 1
	8,540	Toxic	10 ± 2[b]	Toxic	7 ± 0[b]	6 ± 2[b]	4 ± 1[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,507 ± 41	1,188 ± 34	119 ± 11	144 ± 11	100 ± 14	138 ± 7
TA1537							
	0	4 ± 2		6 ± 1	6 ± 1	3 ± 0	7 ± 1
	85.4	8 ± 2		7 ± 1	7 ± 2	4 ± 2	8 ± 1
	284.7	6 ± 2		5 ± 1	8 ± 2	3 ± 2	6 ± 1
	854	7 ± 2		5 ± 1	7 ± 2	4 ± 2	6 ± 1
	2,846	6 ± 1		7 ± 1	7 ± 1	6 ± 1	9 ± 1
	8,540	3 ± 1[b]		5 ± 1	7 ± 1[b]	9 ± 8[b]	4 ± 1[b]
Trial summary		Negative		Negative	Negative	Negative	Negative
Positive control		410 ± 28		344 ± 25	233 ± 15	166 ± 10	193 ± 5

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 30% Hamster S9	With 10% Rat S9	With 30% Rat S9
Study performed at BioReliance Corporation							
TA100							
	0	127 ± 6	101 ± 9	187 ± 16	104 ± 6	155 ± 9	140 ± 18
	100	134 ± 12	119 ± 8	156 ± 11	128 ± 9	167 ± 17	137 ± 5
	333	131 ± 12	91 ± 3	149 ± 7	110 ± 16	159 ± 6	132 ± 27
	1,000	125 ± 4	120 ± 7	160 ± 15	115 ± 9	141 ± 6	129 ± 6
	3,333	123 ± 5	10 ± 4[b]	145 ± 6	98 ± 4	149 ± 9	133 ± 5
	10,000	107 ± 12	8 ± 4[b]	Toxic	93 ± 4	Toxic	4 ± 4[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		361 ± 14	338 ± 21	432 ± 11	664 ± 117	532 ± 72	332 ± 25
TA97							
	0	81 ± 1	212 ± 4[d]	228 ± 6	99 ± 9	232 ± 6	128 ± 13
	100	94 ± 6	175 ± 11	248 ± 7	139 ± 14	188 ± 12	150 ± 14
	333	92 ± 6	224 ± 22	206 ± 27	112 ± 8	216 ± 9	133 ± 12
	1,000	91 ± 14	197 ± 4	234 ± 2	116 ± 6	203 ± 14	123 ± 8
	3,333	85 ± 13	160 ± 4	140 ± 10	100 ± 9	186 ± 18	122 ± 7
	10,000	25 ± 15[b]	Toxic	89 ± 43[b]	74 ± 12[b]	114 ± 7[b]	79 ± 10[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		329 ± 7	665 ± 23	1,381 ± 74	415 ± 54	806 ± 88	314 ± 5

		Without S9	Without S9	Without S9			
TA98							
	0	12 ± 4	18 ± 2	11 ± 2			
	100	11 ± 2	23 ± 1				
	333	13 ± 2	16 ± 1	14 ± 3			
	1,000	10 ± 0	19 ± 3	11 ± 1			
	3,333	10 ± 1	18 ± 2	10 ± 3			
	6,667			12 ± 1			
	10,000	4 ± 4[b]	Toxic	11 ± 3			
Trial summary		Negative	Negative	Negative			
Positive control		162 ± 16	200 ± 7	195 ± 16			

		With 10% Hamster S9	With 30% Hamster S9	With 10% Rat S9	With 30% Rat S9		
TA98 (continued)	0	23 ± 2	26 ± 5	25 ± 3	23 ± 4		
	100	22 ± 3	27 ± 5	21 ± 3	24 ± 3		
	333	27 ± 4	28 ± 2	20 ± 2	25 ± 5		
	1,000	27 ± 1	28 ± 4	17 ± 2	20 ± 0		
	3,333	27 ± 8	33 ± 4	24 ± 0	18 ± 3		
	10,000	19 ± 8[b]	19 ± 2[b]	Toxic	0 ± 0[b]		
Trial summary		Negative	Negative	Negative	Negative		
Positive control		325 ± 32	300 ± 71	253 ± 70	243 ± 32		

Strain	Dose (µg/plate)	Without S9	Without S9	With 10% Hamster S9	With 30% Hamster S9	With 10% Rat S9	With 30% Rat S9
Study performed at BioReliance Corporation (cont'd)							
TA1535							
	0	14 ± 0	18 ± 1	13 ± 2	15 ± 0	10 ± 2	12 ± 1
	100	12 ± 3	18 ± 1	13 ± 2	10 ± 1	14 ± 2	13 ± 3
	333	15 ± 3	18 ± 4	15 ± 1	9 ± 2	16 ± 3	11 ± 1
	1,000	12 ± 1	18 ± 1	13 ± 4	10 ± 2	17 ± 3	12 ± 4
	3,333	11 ± 2	14 ± 2	11 ± 1	10 ± 1	12 ± 3	14 ± 1
	10,000	10 ± 2[b]	17 ± 3[b]	11 ± 3[b]	5 ± 4[b]	9 ± 2[b]	5 ± 3[b]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		134 ± 30	401 ± 55	53 ± 5	109 ± 8	125 ± 14	34 ± 5

[a]Data are presented as revertants/plate (mean ± standard error) from three plates. The detailed protocol is presented by Zeiger et al.[61]. 0 µg/plate was the solvent control.
[b]Slight toxicity.
[c]The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d]Contamination.

Table E-2. Mutagenicity of β-Picoline in Bacterial Tester Strains[a]

Strain	Dose (μg/plate)	Without S9	Without S9	With 10% Rat S9	With 10% Rat S9
TA100					
	0	84 ± 4	76 ± 4	92 ± 8	89 ± 3
	1,000	82 ± 2	76 ± 3	99 ± 3	79 ± 4
	2,500	84 ± 5	74 ± 4	91 ± 8	84 ± 0
	5,000	75 ± 4	71 ± 6	83 ± 5	74 ± 3
	7,500	72 ± 5	63 ± 7	69 ± 5	68 ± 2
	10,000	68 ± 2	64 ± 6	62 ± 1	68 ± 4
Trial summary		Negative	Negative	Negative	Negative
Positive control[b]		416 ± 24	380 ± 37	1,141 ± 31	899 ± 29
TA98					
	0	17 ± 4	18 ± 1	37 ± 1	30 ± 2
	1,000	21 ± 2	18 ± 2	28 ± 1	30 ± 3
	2,500	21 ± 2	14 ± 0	27 ± 1	30 ± 6
	5,000	19 ± 0	13 ± 2	29 ± 4	21 ± 2
	7,500	17 ± 2	12 ± 0	22 ± 3	18 ± 3
	10,000	14 ± 2	10 ± 1	15 ± 1	12 ± 2
Trial summary		Negative	Negative	Negative	Negative
Positive control		604 ± 46	512 ± 24	1,164 ± 37	1,258 ± 28
Escherichia coli WP2 uvrA/pKM101 (analogous to TA102)					
	0	272 ± 12	211 ± 3	312 ± 18	236 ± 1
	100	291 ± 21	270 ± 19	345 ± 13	291 ± 3
	500	310 ± 23	297 ± 11	342 ± 7	269 ± 21
	1,500	275 ± 16	201 ± 20	254 ± 1	218 ± 11
	3,000	73 ± 8	65 ± 7	20 ± 1	32 ± 1
	5,000	53 ± 1	50 ± 3	7 ± 0	10 ± 0
Trial summary		Negative	Negative	Negative	Negative
Positive control		1,880 ± 61	1,711 ± 62	1,064 ± 33	1,010 ± 31

[a]Study was performed at SITEK Research Laboratories using lot no. 11108CI (same lot used in 3-month and 2-year studies). Data are presented as revertants/plate (mean ± standard error) from three plates. 0 μg/plate was the solvent control.
[b]The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (*E. coli*). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E-3. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of β-Picoline in Drinking Water for Three Months[a]

	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Water[d]	0	5	2.50 ± 0.52		3.82 ± 0.25
β-Picoline	78	5	2.20 ± 0.73	0.6693	3.92 ± 0.27
	156	5	2.10 ± 0.37	0.7226	3.78 ± 0.34
	312	5	2.60 ± 0.33	0.4442	3.50 ± 0.18
	625	5	2.90 ± 0.78	0.2929	3.48 ± 0.25
	1,250	5	2.60 ± 0.58	0.4442	3.66 ± 0.19
			P = 0.245[e]		
Female					
Water	0	5	2.60 ± 0.37		3.98 ± 0.55
β-Picoline	78	5	1.90 ± 0.58	0.8519	4.04 ± 0.34
	156	5	2.00 ± 0.22	0.8121	3.30 ± 0.26
	312	5	1.30 ± 0.44	0.9814	3.62 ± 0.30
	625	5	2.10 ± 0.40	0.7674	3.76 ± 0.21
	1,250	5	2.60 ± 0.24	0.5000	3.68 ± 0.61
			P = 0.211		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[62]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the control group; exposed group values are significant at $P \leq 0.005$.
[d]Control.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

Appendix F. Clinical Pathology Results

Tables

Table F-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Male						
n	10	10	10	10	10	10
Hematology						
Hematocrit (auto) (%)						
Day 4	46.1 ± 0.6	45.0 ± 0.7	46.1 ± 0.6	45.9 ± 0.7	47.8 ± 0.7	50.6 ± 1.0**
Day 23	50.1 ± 0.7	52.0 ± 0.8	51.2 ± 0.6	49.5 ± 0.2	50.9 ± 0.5	49.9 ± 0.6
Week 13	47.9 ± 0.3	47.7 ± 0.3	47.8 ± 0.3	47.0 ± 0.4	47.2 ± 0.3	47.0 ± 0.6
Hemoglobin (g/dL)						
Day 4	13.7 ± 0.2	13.4 ± 0.2	13.6 ± 0.2	13.7 ± 0.2	14.2 ± 0.2	15.0 ± 0.3**
Day 23	15.0 ± 0.2	15.6 ± 0.2	15.6 ± 0.2	15.1 ± 0.1	15.3 ± 0.2	15.4 ± 0.2
Week 13	15.1 ± 0.1	14.9 ± 0.1	15.0 ± 0.1	14.8 ± 0.2	15.0 ± 0.1	14.8 ± 0.1
Erythrocytes (10^6/μL)						
Day 4	7.36 ± 0.09	7.08 ± 0.12	7.29 ± 0.09	7.35 ± 0.13	7.64 ± 0.14	8.06 ± 0.16*
Day 23	7.80 ± 0.11	8.08 ± 0.13	8.05 ± 0.08	7.73 ± 0.04	7.91 ± 0.08	8.09 ± 0.08
Week 13	9.09 ± 0.05	9.06 ± 0.05	9.00 ± 0.05	8.84 ± 0.07**	8.89 ± 0.05**	8.84 ± 0.10*
Reticulocytes (10^6/μL)						
Day 4	0.52 ± 0.02	0.56 ± 0.02	0.57 ± 0.03	0.38 ± 0.02	0.49 ± 0.02	0.39 ± 0.01**
Day 23	0.32 ± 0.02	0.30 ± 0.01	0.27 ± 0.01	0.33 ± 0.01	0.33 ± 0.07	0.19 ± 0.04
Week 13	0.24 ± 0.05	0.24 ± 0.05	0.23 ± 0.08	0.24 ± 0.06	0.23 ± 0.08	0.22 ± 0.10
Mean cell volume (fL)						
Day 4	62.6 ± 0.4	63.6 ± 0.3	63.2 ± 0.3	62.5 ± 0.3	62.6 ± 0.4	62.8 ± 0.2
Day 23	64.3 ± 0.3	64.3 ± 0.4	63.7 ± 0.3	63.9 ± 0.2	64.3 ± 0.4	61.6 ± 0.5**
Week 13	52.7 ± 0.2	52.7 ± 0.2	53.2 ± 0.2	53.1 ± 0.2	53.0 ± 0.2	53.2 ± 0.3
Mean cell hemoglobin (pg)						
Day 4	18.6 ± 0.1	18.9 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.6 ± 0.2	18.6 ± 0.1
Day 23	19.3 ± 0.1	19.3 ± 0.1	19.3 ± 0.1	19.5 ± 0.1	19.4 ± 0.1	19.0 ± 0.1
Week 13	16.6 ± 0.1	16.5 ± 0.1	16.7 ± 0.1	16.7 ± 0.1	16.8 ± 0.1	16.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	29.6 ± 0.2	29.8 ± 0.1	29.6 ± 0.2	29.8 ± 0.2	29.7 ± 0.3	29.6 ± 0.1
Day 23	30.0 ± 0.1	30.0 ± 0.1	30.4 ± 0.2	30.5 ± 0.2	30.1 ± 0.2	30.9 ± 0.2**
Week 13	31.4 ± 0.1	31.3 ± 0.2	31.4 ± 0.1	31.5 ± 0.2	31.7 ± 0.2	31.6 ± 0.2
Platelets (10^3/μL)						
Day 4	1,088.7 ± 29.3	1,091.5 ± 30.7	1,128.2 ± 32.3	1,030.7 ± 41.7	1,057.9 ± 66.1	1,196.5 ± 31.1
Day 23	926.8 ± 21.4	890.1 ± 15.4	848.2 ± 15.0**	832.1 ± 15.5**	858.1 ± 25.7*	724.0 ± 43.2**

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Week 13	623.4 ± 24.4	651.9 ± 25.5	616.3 ± 22.6	600.7 ± 23.1	538.0 ± 49.4	589.5 ± 29.2
Leukocytes (10^3/μL)						
Day 4	10.17 ± 0.27	8.66 ± 0.49	9.92 ± 0.38	10.25 ± 0.50	9.01 ± 0.59	10.08 ± 0.45
Day 23	9.77 ± 0.35	9.61 ± 0.36	10.14 ± 0.40	9.70 ± 0.49	10.27 ± 0.40	8.63 ± 0.42
Week 13	7.79 ± 0.45	8.15 ± 0.37	7.69 ± 0.51	7.75 ± 0.27	8.11 ± 0.30	8.52 ± 0.43
Segmented neutrophils (10^3/μL)						
Day 4	1.13 ± 0.05	0.97 ± 0.06	1.15 ± 0.05	1.10 ± 0.05	1.05 ± 0.05	1.12 ± 0.05
Day 23	1.10 ± 0.09	1.03 ± 0.04	1.02 ± 0.04	1.21 ± 0.06	1.09 ± 0.07	1.08 ± 0.13
Week 13	1.35 ± 0.09	1.43 ± 0.14	1.34 ± 0.10	1.38 ± 0.07	1.82 ± 0.14*	1.67 ± 0.10*
Lymphocytes (10^3/μL)						
Day 4	8.66 ± 0.24	7.38 ± 0.43	8.40 ± 0.37	8.76 ± 0.44	7.62 ± 0.55	8.63 ± 0.42
Day 23	8.39 ± 0.37	8.31 ± 0.33	8.85 ± 0.36	8.23 ± 0.43	8.92 ± 0.33	7.31 ± 0.33
Week 13	6.08 ± 0.37	6.38 ± 0.25	6.01 ± 0.47	6.04 ± 0.29	5.91 ± 0.18	6.47 ± 0.36
Monocytes (10^3/μL)						
Day 4	0.30 ± 0.02	0.24 ± 0.02	0.27 ± 0.02	0.30 ± 0.03	0.24 ± 0.02	0.23 ± 0.02
Day 23	0.17 ± 0.02	0.16 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.16 ± 0.01	0.15 ± 0.01
Week 13	0.21 ± 0.02	0.20 ± 0.02	0.22 ± 0.02	0.19 ± 0.01	0.23 ± 0.02	0.22 ± 0.02
Basophils (10^3/μL)						
Day 4	0.040 ± 0.003	0.041 ± 0.005	0.057 ± 0.012	0.047 ± 0.003	0.038 ± 0.007	0.053 ± 0.006
Day 23	0.041 ± 0.003	0.038 ± 0.005	0.033 ± 0.004	0.040 ± 0.004	0.036 ± 0.003	0.035 ± 0.004
Week 13	0.046 ± 0.005	0.028 ± 0.004*	0.033 ± 0.004	0.034 ± 0.004	0.035 ± 0.006	0.048 ± 0.010
Eosinophils (10^3/μL)						
Day 4	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.07 ± 0.02	0.04 ± 0.00
Day 23	0.08 ± 0.03	0.07 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.01
Week 13	0.11 ± 0.01	0.11 ± 0.02	0.09 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
Clinical Chemistry						
Urea nitrogen (mg/dL)						
Day 4	13.0 ± 0.5	12.5 ± 0.4	12.5 ± 0.3	14.4 ± 0.6	15.2 ± 0.9	16.9 ± 1.1**
Day 23	13.9 ± 0.3	15.6 ± 0.7	16.3 ± 0.5**	16.6 ± 1.1*	17.8 ± 1.2**	17.9 ± 1.4**
Week 13	15.3 ± 0.5	15.7 ± 0.3	15.7 ± 0.5	14.8 ± 0.3	15.9 ± 0.4	15.9 ± 0.3
Creatinine (mg/dL)						
Day 4	0.41 ± 0.01	0.41 ± 0.01	0.43 ± 0.02	0.43 ± 0.02	0.42 ± 0.01	0.43 ± 0.02
Day 23	0.47 ± 0.02	0.47 ± 0.02	0.45 ± 0.02	0.47 ± 0.02	0.46 ± 0.02	0.47 ± 0.02
Week 13	0.56 ± 0.02	0.56 ± 0.02	0.54 ± 0.02	0.56 ± 0.02	0.56 ± 0.02	0.58 ± 0.01
Glucose (mg/dL)						
Week 13	140 ± 3	139 ± 4	146 ± 6	130 ± 4	134 ± 3	136 ± 3

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.8 ± 0.1	6.1 ± 0.1**
Day 23	6.3 ± 0.1	6.4 ± 0.1	6.3 ± 0.1	6.1 ± 0.1	6.1 ± 0.1	5.7 ± 0.1**
Week 13	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1**	6.4 ± 0.1**
Albumin (g/dL)						
Day 4	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.0	4.0 ± 0.0	4.1 ± 0.1	4.4 ± 0.1**
Day 23	4.3 ± 0.1	4.3 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.1 ± 0.1*
Week 13	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.1	4.4 ± 0.0	4.4 ± 0.0*	4.4 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	48 ± 2	52 ± 1	49 ± 2	46 ± 1	45 ± 2	39 ± 2**
Day 23	50 ± 1	55 ± 2	53 ± 2	56 ± 2	50 ± 3	52 ± 3
Week 13	142 ± 16	98 ± 8	126 ± 16	96 ± 8	115 ± 11	80 ± 6**
Alkaline phosphatase (IU/L)						
Day 4	615 ± 15	628 ± 13	637 ± 12	601 ± 15	633 ± 17	647 ± 14
Day 23	461 ± 10	460 ± 11	447 ± 10	474 ± 9	472 ± 7	395 ± 27
Week 13	208 ± 4	200 ± 2	206 ± 3	199 ± 3	204 ± 4	217 ± 4
Creatine kinase (IU/L)						
Day 4	374 ± 36	383 ± 76	441 ± 58	435 ± 61	421 ± 40	407 ± 38
Day 23	290 ± 45	290 ± 51	255 ± 32	266 ± 40	244 ± 23	244 ± 39
Week 13	135 ± 21	161 ± 38	129 ± 19	110 ± 9	135 ± 35	146 ± 26
Sorbitol dehydrogenase (IU/L)						
Day 4	14 ± 0	13 ± 1	13 ± 0	14 ± 0	13 ± 0	15 ± 0
Day 23	16 ± 1	16 ± 1	16 ± 1	16 ± 1	15 ± 1	14 ± 1
Week 13	46 ± 6	32 ± 3	40 ± 5	33 ± 4	44 ± 5	31 ± 2
Bile salts (µmol/L)						
Day 4	7.5 ± 0.8	7.3 ± 0.7	7.8 ± 0.8	10.4 ± 1.0	7.5 ± 0.7	7.5 ± 1.1
Day 23	5.8 ± 1.3	7.7 ± 1.1	4.5 ± 0.6	5.9 ± 0.8	5.3 ± 1.4	5.1 ± 0.7
Week 13	5.9 ± 1.0	6.6 ± 1.3	7.0 ± 1.6	4.9 ± 0.6	6.3 ± 1.7	10.5 ± 1.3
Female						
Hematology						
n						
Day 4	10	10	10	10	10	10
Day 23	10	10	10	10	10	9
Week 13	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 4	47.7 ± 0.7	49.1 ± 1.2	47.9 ± 0.7	48.8 ± 0.8	47.9 ± 0.8	50.0 ± 1.0

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Day 23	48.2 ± 0.6	48.1 ± 0.6	48.6 ± 0.6	49.4 ± 0.7	47.7 ± 0.6	47.6 ± 0.8
Week 13	44.7 ± 0.5	45.3 ± 0.5	45.1 ± 0.5	45.7 ± 0.4	45.3 ± 0.4	43.8 ± 0.4
Hemoglobin (g/dL)						
Day 4	14.1 ± 0.2	14.5 ± 0.3	14.2 ± 0.2	14.3 ± 0.3	14.1 ± 0.2	14.8 ± 0.3
Day 23	15.3 ± 0.2	15.6 ± 0.2	15.4 ± 0.2	15.8 ± 0.2	15.2 ± 0.2	15.0 ± 0.2
Week 13	14.6 ± 0.1	14.8 ± 0.1	14.8 ± 0.2	14.9 ± 0.1	14.7 ± 0.1	14.4 ± 0.1
Erythrocytes (10^6/μL)						
Day 4	7.58 ± 0.12	7.80 ± 0.18	7.70 ± 0.12	7.72 ± 0.15	7.67 ± 0.15	8.06 ± 0.16
Day 23	8.13 ± 0.12	8.17 ± 0.08	8.21 ± 0.08	8.32 ± 0.13	8.03 ± 0.10	8.05 ± 0.12
Week 13	8.13 ± 0.07	8.26 ± 0.09	8.17 ± 0.09	8.29 ± 0.08	8.19 ± 0.07	7.99 ± 0.06
Reticulocytes (10^6/μL)						
Day 4	0.45 ± 0.02	0.46 ± 0.02	0.42 ± 0.03	0.45 ± 0.03	0.37 ± 0.02*	0.34 ± 0.03**
Day 23	0.15 ± 0.01	0.17 ± 0.00	0.16 ± 0.01	0.19 ± 0.01**	0.20 ± 0.01**	0.21 ± 0.01**
Week 13	0.19 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.20 ± 0.01
Mean cell volume (fL)						
Day 4	62.9 ± 0.5	63.0 ± 0.3	62.2 ± 0.3	63.3 ± 0.4	62.5 ± 0.4	62.1 ± 0.3
Day 23	59.3 ± 0.3	58.9 ± 0.3	59.2 ± 0.3	59.5 ± 0.3	59.4 ± 0.3	59.1 ± 0.4
Week 13	55.0 ± 0.1	54.9 ± 0.1	55.2 ± 0.1	55.1 ± 0.2	55.3 ± 0.2	54.9 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	18.6 ± 0.1	18.6 ± 0.1	18.4 ± 0.1	18.5 ± 0.1	18.4 ± 0.1	18.4 ± 0.1
Day 23	18.9 ± 0.1	19.1 ± 0.1	18.8 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	18.6 ± 0.1
Week 13	18.0 ± 0.1	18.0 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	18.0 ± 0.1	18.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	29.6 ± 0.2	29.6 ± 0.1	29.6 ± 0.1	29.2 ± 0.2	29.4 ± 0.2	29.6 ± 0.2
Day 23	31.8 ± 0.2	32.4 ± 0.2	31.8 ± 0.2	32.0 ± 0.1	31.8 ± 0.2	31.5 ± 0.2
Week 13	32.7 ± 0.1	32.8 ± 0.1	32.7 ± 0.2	32.6 ± 0.2	32.5 ± 0.1	32.8 ± 0.1
Platelets (10^3/μL)						
Day 4	1,036.3 ± 45.2	1,041.6 ± 16.5	929.9 ± 48.5	1,044.3 ± 37.1	1,033.7 ± 34.2	1,040.4 ± 27.8
Day 23	789.5 ± 56.4	772.0 ± 26.7	722.9 ± 61.4	763.2 ± 56.5	817.7 ± 22.9	839.2 ± 46.9
Week 13	630.3 ± 34.5	594.4 ± 34.0	648.0 ± 19.5	665.6 ± 20.6	562.8 ± 33.7	606.6 ± 33.5
Leukocytes (10^3/μL)						
Day 4	10.51 ± 0.74	10.43 ± 0.29	10.05 ± 0.45	10.57 ± 0.37	10.75 ± 0.26	10.89 ± 0.54
Day 23	9.11 ± 0.58	9.15 ± 0.59	6.86 ± 0.37*	8.64 ± 0.40	7.88 ± 0.69	7.65 ± 0.32
Week 13	7.14 ± 0.24	6.10 ± 0.44	6.86 ± 0.13	5.84 ± 0.37*	6.20 ± 0.22	7.38 ± 0.36
Segmented neutrophils (10^3/μL)						
Day 4	0.96 ± 0.10	1.00 ± 0.06	0.92 ± 0.06	1.14 ± 0.08	1.00 ± 0.07	0.88 ± 0.03

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Day 23	0.99 ± 0.14	1.05 ± 0.13	0.73 ± 0.06	0.91 ± 0.09	0.90 ± 0.10	0.95 ± 0.07
Week 13	1.41 ± 0.09	1.04 ± 0.11	1.38 ± 0.09	1.18 ± 0.11	1.24 ± 0.05	1.80 ± 0.13
Lymphocytes (10^3/μL)						
Day 4	9.20 ± 0.67	9.07 ± 0.27	8.82 ± 0.42	9.05 ± 0.32	9.36 ± 0.29	9.64 ± 0.52
Day 23	7.89 ± 0.46	7.84 ± 0.55	5.94 ± 0.32*	7.50 ± 0.35	6.79 ± 0.60	6.49 ± 0.36
Week 13	5.38 ± 0.24	4.75 ± 0.36	5.15 ± 0.08	4.37 ± 0.26*	4.67 ± 0.20	5.25 ± 0.27
Monocytes (10^3/μL)						
Day 4	0.23 ± 0.02	0.25 ± 0.01	0.21 ± 0.02	0.26 ± 0.02	0.26 ± 0.02	0.26 ± 0.01
Day 23	0.13 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.12 ± 0.01
Week 13	0.21 ± 0.01	0.19 ± 0.03	0.20 ± 0.02	0.17 ± 0.02	0.14 ± 0.01*	0.19 ± 0.02
Basophils (10^3/μL)						
Day 4	0.062 ± 0.006	0.049 ± 0.003	0.053 ± 0.005	0.064 ± 0.006	0.054 ± 0.006	0.056 ± 0.005
Day 23	0.030 ± 0.004	0.034 ± 0.006	0.030 ± 0.005	0.037 ± 0.004	0.028 ± 0.004	0.021 ± 0.004
Week 13	0.025 ± 0.003	0.025 ± 0.006	0.026 ± 0.005	0.030 ± 0.006	0.022 ± 0.004	0.032 ± 0.004
Eosinophils (10^3/μL)						
Day 4	0.07 ± 0.02	0.07 ± 0.02	0.05 ± 0.01	0.07 ± 0.03	0.08 ± 0.03	0.07 ± 0.02
Day 23	0.07 ± 0.02	0.11 ± 0.03	0.06 ± 0.01	0.08 ± 0.02	0.06 ± 0.01	0.06 ± 0.02
Week 13	0.11 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	13.9 ± 0.5	12.5 ± 0.3	13.4 ± 0.7	13.1 ± 0.4	14.2 ± 0.4	15.0 ± 0.3
Day 23	17.8 ± 0.6	18.1 ± 0.4	15.6 ± 0.6	17.6 ± 0.4	17.9 ± 0.4	16.0 ± 0.3
Week 13	15.3 ± 0.5	15.0 ± 0.2	14.7 ± 0.4	15.4 ± 0.4	15.4 ± 0.5	15.4 ± 0.4
Creatinine (mg/dL)						
Day 4	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02	0.42 ± 0.01	0.43 ± 0.02	0.43 ± 0.02
Day 23	0.51 ± 0.01	0.51 ± 0.01	0.52 ± 0.01	0.53 ± 0.02	0.51 ± 0.01	0.50 ± 0.00
Week 13	0.52 ± 0.01	0.51 ± 0.01	0.50 ± 0.00	0.50 ± 0.00	0.51 ± 0.01	0.51 ± 0.01
Glucose (mg/dL)						
Week 13	141 ± 4	139 ± 3	148 ± 7	147 ± 5	143 ± 6	144 ± 5
Total protein (g/dL)						
Day 4	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.1 ± 0.1
Day 23	6.6 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.6 ± 0.1	6.5 ± 0.1
Week 13	6.4 ± 0.1	6.8 ± 0.2	6.4 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.0 ± 0.1*
Albumin (g/dL)						
Day 4	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.3 ± 0.1

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Day 23	4.7 ± 0.1	4.8 ± 0.1	4.7 ± 0.0	4.8 ± 0.0	4.7 ± 0.0	4.7 ± 0.1
Week 13	4.5 ± 0.1	4.9 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	4.4 ± 0.0	4.4 ± 0.0
Alanine aminotransferase (IU/L)						
Day 4	41 ± 1	42 ± 2	43 ± 2	43 ± 2	40 ± 1	36 ± 3
Day 23	36 ± 2	37 ± 1	40 ± 1*	39 ± 1	38 ± 1	38 ± 1
Week 13	59 ± 4	62 ± 4	49 ± 4	52 ± 4	50 ± 3	54 ± 2
Alkaline phosphatase (IU/L)						
Day 4	520 ± 12	518 ± 10	517 ± 12	524 ± 10	510 ± 12	525 ± 10
Day 23	344 ± 8	341 ± 7	347 ± 6	353 ± 4	376 ± 8*	391 ± 9**
Week 13	157 ± 7	136 ± 2	145 ± 5	149 ± 5	158 ± 4	166 ± 5
Creatine kinase (IU/L)						
Day 4	408 ± 47	342 ± 31	379 ± 72	376 ± 57	368 ± 45	436 ± 52
Day 23	286 ± 63	279 ± 43	252 ± 38	218 ± 28	202 ± 22	298 ± 42
Week 13	149 ± 33	81 ± 10	137 ± 31	135 ± 39	164 ± 35	111 ± 21
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 1	15 ± 1	14 ± 1	15 ± 0	13 ± 1	13 ± 1
Day 23	13 ± 1	14 ± 0	13 ± 1	13 ± 0	13 ± 1	14 ± 1
Week 13	19 ± 2	16 ± 1	15 ± 1	16 ± 1	17 ± 1	15 ± 1
Bile salts (µmol/L)						
Day 4	5.2 ± 0.7	6.2 ± 0.9	5.8 ± 0.8	6.4 ± 0.7	5.0 ± 0.5	5.3 ± 0.4
Day 23	5.8 ± 0.8	4.1 ± 0.4	5.1 ± 0.6	6.4 ± 1.2	4.9 ± 0.5	4.8 ± 0.5
Week 13	7.4 ± 1.3	7.7 ± 1.1	11.4 ± 1.0	9.1 ± 2.0	10.5 ± 3.1	7.9 ± 1.3

*Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
**Significantly different ($P \leq 0.01$) from the control group by Shirley's test.
[a]Mean ± standard error. Statistical tests were performed on unrounded data.

Table F-2. Hematology Data for Mice in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Male						
n	10	10	10	10	10	10
Hematocrit (auto) (%)	47.8 ± 0.4	47.6 ± 0.5	47.4 ± 0.6	48.6 ± 0.7	46.3 ± 0.4	48.1 ± 0.5
Hemoglobin (g/dL)	16.2 ± 0.2	16.2 ± 0.2	15.9 ± 0.2	16.5 ± 0.2	15.8 ± 0.2	16.4 ± 0.2
Erythrocytes (10^6/μL)	10.91 ± 0.08	10.83 ± 0.11	10.73 ± 0.15	11.04 ± 0.15	10.48 ± 0.11	10.95 ± 0.13
Reticulocytes (10^6/μL)	0.28 ± 0.01	0.28 ± 0.01	0.27 ± 0.00	0.27 ± 0.01	0.27 ± 0.00	0.27 ± 0.00
Mean cell volume (fL)	43.8 ± 0.1	44.0 ± 0.2	44.2 ± 0.1	44.0 ± 0.2	44.2 ± 0.2	43.9 ± 0.2
Mean cell hemoglobin (pg)	14.9 ± 0.0	15.0 ± 0.1	14.8 ± 0.1	15.0 ± 0.1	15.1 ± 0.1*	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.9 ± 0.1	34.1 ± 0.2	33.6 ± 0.2	34.0 ± 0.2	34.1 ± 0.2	34.0 ± 0.1
Platelets (10^3/μL)	816.3 ± 55.5	843.1 ± 46.0	922.0 ± 41.2	772.8 ± 44.4	916.3 ± 43.4	764.9 ± 31.8
Leukocytes (10^3/μL)	5.20 ± 0.32	4.82 ± 0.40	5.83 ± 0.16	5.09 ± 0.49	4.61 ± 0.42	5.15 ± 0.53
Segmented neutrophils (10^3/μL)	0.87 ± 0.09	0.67 ± 0.08	0.79 ± 0.10	0.73 ± 0.11	0.81 ± 0.05	0.66 ± 0.07
Lymphocytes (10^3/μL)	4.04 ± 0.27	3.86 ± 0.32	4.68 ± 0.14	4.03 ± 0.41	3.54 ± 0.37	4.20 ± 0.47
Monocytes (10^3/μL)	0.19 ± 0.02	0.21 ± 0.05	0.28 ± 0.04	0.20 ± 0.02	0.18 ± 0.05	0.22 ± 0.04
Basophils (10^3/μL)	0.002 ± 0.001	0.014 ± 0.007	0.002 ± 0.001	0.004 ± 0.004	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.11 ± 0.03	0.07 ± 0.02	0.08 ± 0.02	0.13 ± 0.03	0.08 ± 0.02	0.07 ± 0.02
Female						
n	10	9	10	10	10	10
Hematocrit (auto) (%)	48.3 ± 0.6	46.4 ± 0.4	45.9 ± 0.6*	46.8 ± 0.4	47.7 ± 0.7	45.8 ± 0.3**
Hemoglobin (g/dL)	16.5 ± 0.2	16.0 ± 0.2	15.7 ± 0.2*	16.1 ± 0.1	16.4 ± 0.2	15.8 ± 0.1*
Erythrocytes (10^6/μL)	10.93 ± 0.14	10.46 ± 0.08	10.35 ± 0.14*	10.56 ± 0.08	10.79 ± 0.15	10.37 ± 0.07*
Reticulocytes (10^6/μL)	0.30 ± 0.02	0.34 ± 0.04	0.29 ± 0.02	0.29 ± 0.02	0.28 ± 0.01	0.27 ± 0.02
Mean cell volume (fL)	44.2 ± 0.2	44.4 ± 0.2	44.3 ± 0.2	44.3 ± 0.1	44.2 ± 0.2	44.2 ± 0.2
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.2 ± 0.0	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.3 ± 0.1	34.5 ± 0.1	34.3 ± 0.1	34.4 ± 0.2	34.4 ± 0.1	34.4 ± 0.2
Platelets (10^3/μL)	668.5 ± 66.7	723.6 ± 51.3	842.7 ± 73.4	722.8 ± 41.1	701.9 ± 48.4	802.3 ± 67.4
Leukocytes (10^3/μL)	5.29 ± 0.38	4.11 ± 0.35	4.71 ± 0.26	5.13 ± 0.31	4.82 ± 0.23	5.37 ± 0.27
Segmented neutrophils (10^3/μL)	0.66 ± 0.06	0.54 ± 0.06	0.63 ± 0.09	0.86 ± 0.11	0.76 ± 0.09	0.67 ± 0.10
Lymphocytes (10^3/μL)	4.29 ± 0.34	3.25 ± 0.35	3.73 ± 0.25	3.93 ± 0.25	3.71 ± 0.18	4.29 ± 0.20
Monocytes (10^3/μL)	0.25 ± 0.04	0.21 ± 0.06	0.23 ± 0.06	0.18 ± 0.04	0.19 ± 0.05	0.26 ± 0.06
Basophils (10^3/μL)	0.007 ± 0.005	0.003 ± 0.002	0.004 ± 0.002	0.005 ± 0.002	0.007 ± 0.005	0.008 ± 0.005
Eosinophils (10^3/μL)	0.08 ± 0.02	0.10 ± 0.03	0.13 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.14 ± 0.03

*Significantly different ($P \leq 0.05$) from the control group by Dunn's test.
**$P \leq 0.01$.
[a]Mean ± standard error. Statistical tests were performed on unrounded data.

Appendix G. Organ Weights and Organ-Weight-to-Body-Weight Ratios

Tables

Table G-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	356 ± 5	349 ± 7	352 ± 4	356 ± 4	332 ± 4**	298 ± 5**
Heart						
Absolute	0.97 ± 0.01	0.94 ± 0.02	0.98 ± 0.01	0.96 ± 0.02	0.92 ± 0.03*	0.82 ± 0.02**
Relative	2.735 ± 0.032	2.699 ± 0.036	2.779 ± 0.036	2.711 ± 0.049	2.771 ± 0.060	2.769 ± 0.040
R. Kidney						
Absolute	1.09 ± 0.02	1.05 ± 0.02	1.08 ± 0.01	1.14 ± 0.02	1.10 ± 0.02	1.07 ± 0.03
Relative	3.046 ± 0.060	3.018 ± 0.037	3.055 ± 0.034	3.196 ± 0.033*	3.324 ± 0.048**	3.597 ± 0.062**
Liver						
Absolute	12.73 ± 0.36	12.22 ± 0.32	12.63 ± 0.24	12.47 ± 0.23	11.52 ± 0.16**	10.38 ± 0.23**
Relative	35.671 ± 0.686	35.001 ± 0.436	35.848 ± 0.440	35.063 ± 0.415	34.763 ± 0.393	34.894 ± 0.432
Lung						
Absolute	1.98 ± 0.08	1.89 ± 0.07	1.82 ± 0.05	1.92 ± 0.07	1.80 ± 0.07	1.58 ± 0.06**
Relative	5.560 ± 0.226	5.419 ± 0.183	5.186 ± 0.178	5.410 ± 0.186	5.425 ± 0.226	5.296 ± 0.160
R. Testis						
Absolute	1.430 ± 0.020	1.423 ± 0.022	1.422 ± 0.015	1.428 ± 0.014	1.405 ± 0.020	1.344 ± 0.020**
Relative	4.016 ± 0.060	4.082 ± 0.046	4.042 ± 0.065	4.022 ± 0.065	4.243 ± 0.072**	4.520 ± 0.029**
Thymus						
Absolute	0.311 ± 0.013	0.314 ± 0.014	0.318 ± 0.015	0.292 ± 0.008	0.288 ± 0.013	0.262 ± 0.012*
Relative	0.874 ± 0.039	0.901 ± 0.037	0.903 ± 0.038	0.824 ± 0.030	0.870 ± 0.042	0.884 ± 0.045

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Female						
Necropsy body wt	207 ± 5	206 ± 2	205 ± 4	205 ± 3	192 ± 3**	177 ± 4**
Heart						
Absolute	0.64 ± 0.01	0.66 ± 0.01	0.66 ± 0.02	0.64 ± 0.01	0.60 ± 0.02	0.55 ± 0.01**
Relative	3.082 ± 0.069	3.195 ± 0.070	3.202 ± 0.054	3.114 ± 0.049	3.126 ± 0.072	3.118 ± 0.056
R. Kidney						
Absolute	0.69 ± 0.02	0.74 ± 0.02	0.72 ± 0.01	0.72 ± 0.01	0.71 ± 0.02	0.69 ± 0.02
Relative	3.358 ± 0.076	3.563 ± 0.048	3.520 ± 0.052	3.517 ± 0.055	3.702 ± 0.056**	3.894 ± 0.078**
Liver						
Absolute	6.69 ± 0.21	6.62 ± 0.15	6.37 ± 0.19	6.28 ± 0.13	6.02 ± 0.13**	5.42 ± 0.09**
Relative	32.295 ± 0.521	32.124 ± 0.543	31.040 ± 0.433	30.585 ± 0.369*	31.289 ± 0.290*	30.651 ± 0.408*
Lung						
Absolute	1.29 ± 0.03	1.24 ± 0.03	1.23 ± 0.04	1.26 ± 0.03	1.22 ± 0.06	1.11 ± 0.03**
Relative	6.274 ± 0.200	6.008 ± 0.127	6.004 ± 0.161	6.147 ± 0.163	6.368 ± 0.339	6.267 ± 0.098
Thymus						
Absolute	0.281 ± 0.012	0.253 ± 0.010	0.259 ± 0.010	0.246 ± 0.010*	0.231 ± 0.012**	0.216 ± 0.008**
Relative	1.358 ± 0.049	1.232 ± 0.053	1.262 ± 0.033	1.200 ± 0.057	1.201 ± 0.050	1.219 ± 0.039

*Significantly different ($P \leq 0.05$) from the control group by Williams' test.
**Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Table G-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.5 ± 1.3	39.5 ± 0.8	39.9 ± 1.2	39.9 ± 1.3	39.6 ± 1.1	37.9 ± 1.0
Heart						
Absolute	0.20 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
Relative	5.346 ± 0.284	5.625 ± 0.165	5.385 ± 0.194	5.527 ± 0.244	5.625 ± 0.258	5.951 ± 0.306
R. Kidney						
Absolute	0.30 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
Relative	7.923 ± 0.264	8.252 ± 0.193	8.318 ± 0.237	8.289 ± 0.239	7.982 ± 0.185	8.112 ± 0.215
Liver						
Absolute	1.79 ± 0.05	1.90 ± 0.05	1.97 ± 0.08	2.01 ± 0.09	2.02 ± 0.09	1.84 ± 0.05
Relative	46.637 ± 0.816	48.228 ± 0.895	49.402 ± 0.715	50.212 ± 1.084*	50.851 ± 0.936**	48.599 ± 0.589
Lung						
Absolute	0.31 ± 0.02	0.32 ± 0.01	0.28 ± 0.01	0.34 ± 0.01	0.34 ± 0.03	0.34 ± 0.01
Relative	8.100 ± 0.643	8.163 ± 0.272	7.136 ± 0.253	8.603 ± 0.312	8.430 ± 0.646	9.113 ± 0.379
R. Testis						
Absolute	0.115 ± 0.003	0.118 ± 0.003	0.119 ± 0.002	0.115 ± 0.002	0.112 ± 0.003	0.115 ± 0.002
Relative	3.020 ± 0.130	3.000 ± 0.074	3.003 ± 0.086	2.905 ± 0.107	2.846 ± 0.098	3.055 ± 0.095
Thymus						
Absolute	0.049 ± 0.004	0.050 ± 0.003	0.058 ± 0.005	0.054 ± 0.003	0.054 ± 0.003	0.046 ± 0.004
Relative	1.276 ± 0.107	1.264 ± 0.058	1.439 ± 0.093	1.353 ± 0.059	1.371 ± 0.090	1.212 ± 0.088

	0 mg/L	78 mg/L	156 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Female						
Necropsy body wt	27.2 ± 0.9	27.6 ± 1.0	30.1 ± 1.0	28.0 ± 1.1	27.6 ± 0.5	27.9 ± 0.9
Heart						
Absolute	0.16 ± 0.00	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Relative	5.902 ± 0.256	6.072 ± 0.310	5.474 ± 0.232	5.714 ± 0.262	6.146 ± 0.184	5.776 ± 0.206
R. Kidney						
Absolute	0.17 ± 0.00	0.18 ± 0.00	0.19 ± 0.00	0.17 ± 0.01	0.17 ± 0.00	0.17 ± 0.01
Relative	6.403 ± 0.166	6.409 ± 0.160	6.316 ± 0.243	6.142 ± 0.147	6.193 ± 0.138	6.274 ± 0.125
Liver						
Absolute	1.17 ± 0.04	1.27 ± 0.04	1.36 ± 0.03**	1.25 ± 0.05	1.24 ± 0.02	1.26 ± 0.05
Relative	43.251 ± 1.075	46.058 ± 0.702	45.281 ± 0.899	44.763 ± 0.798	44.928 ± 1.015	45.250 ± 1.043
Lung						
Absolute	0.30 ± 0.01	0.29 ± 0.01	0.27 ± 0.02	0.27 ± 0.01	0.28 ± 0.01	0.25 ± 0.02**
Relative	11.005 ± 0.494	10.777 ± 0.500	8.931 ± 0.652*	9.726 ± 0.383*	10.022 ± 0.364*	8.787 ± 0.469**
Thymus						
Absolute	0.055 ± 0.004	0.055 ± 0.004	0.060 ± 0.003	0.049 ± 0.002	0.050 ± 0.001	0.054 ± 0.001
Relative	2.043 ± 0.139	2.026 ± 0.161	2.005 ± 0.071	1.769 ± 0.100	1.807 ± 0.065	1.965 ± 0.075

*Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.
**$P \leq 0.01$.
[a]Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

Appendix H. Reproductive Tissue Evaluations and Estrous Cycle Characterization

Tables

Figures

Table H-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	156 mg/L	312 mg/L	625 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	356 ± 5	352 ± 4	356 ± 4	332 ± 4**
L. Cauda epididymis	0.1458 ± 0.0036	0.1446 ± 0.0036	0.1436 ± 0.0018	0.1455 ± 0.0038
L. Epididymis	0.4315 ± 0.0046	0.4169 ± 0.0090	0.4289 ± 0.0057	0.4265 ± 0.0043
L. Testis	1.5383 ± 0.0107	1.4650 ± 0.0272*	1.4857 ± 0.0156	1.4805 ± 0.0103
Spermatid measurements				
Spermatid heads (10^6/testis)	181.13 ± 5.24	169.50 ± 10.42	167.88 ± 6.28	175.63 ± 5.67
Spermatid heads (10^6/g testis)	131.7 ± 3.9	128.1 ± 6.3	125.0 ± 3.9	134.0 ± 4.3
Epididymal spermatozoal measurements				
Sperm motility (%)	77.2 ± 0.9	77.7 ± 1.2	78.0 ± 1.2	78.8 ± 1.0
Sperm (10^6/cauda epididymis)	113.13 ± 5.98	109.38 ± 4.49	121.25 ± 4.63	106.13 ± 3.73
Sperm (10^6/g cauda epididymis)	777.6 ± 40.9	760.9 ± 36.1	845.0 ± 32.5	730.7 ± 23.8

*Significantly different ($P \leq 0.05$) from the control group by Dunnett's test.
**Significantly different ($P \leq 0.01$) from the control group by Williams' test.
[a]Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (cauda epididymis and epididymis weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table H-2. Estrous Cycle Characterization for Female Rats in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	156 mg/L	312 mg/L	625 mg/L
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	207 ± 5	205 ± 4	205 ± 3	192 ± 3*
Proportion of regular cycling females[b]	6/6	4/6	8/10	8/9
Estrous cycle length (days)	4.8 ± 0.11[c]	5.3 ± 0.33[c]	5.1 ± 0.10	5.1 ± 0.06[d]
Estrous stages (% of cycle)				
Diestrus	70.0	74.2	61.7	65.0
Proestrus	7.5	7.5	10.8	10.8
Estrus	17.5	15.8	25.0	23.3
Metestrus	5.0	2.5	2.5	0.8

*Significantly different ($P \leq 0.05$) from the control group by Williams' test.
[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices indicated a significantly higher probability of extended estrus for female rats in the 312 and 625 mg/L groups compared to the control group.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle was longer than 12 days or unclear in four of 10 animals.
[d]Estrous cycle was longer than 12 days or unclear in one of 10 animals.

Table H-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach in Female Rats Exposed to β-Picoline in Drinking Water for Three Months

Stage	Comparison[a]	P-Value	Trend[b]
Overall Tests	Overall	<0.001	
Overall Tests	Low vs. controls	0.819	N
Overall Tests	Mid vs. controls	<0.001	–
Overall Tests	High vs. controls	<0.001	–
Extended Estrus	Overall	0.347	
Extended Estrus	Low vs. controls	0.836	N
Extended Estrus	Mid vs. controls	0.049	–
Extended Estrus	High vs. controls	0.842	–
Extended Diestrus	Overall	<0.001	
Extended Diestrus	Low vs. controls	0.709	–
Extended Diestrus	Mid vs. controls	<0.001	–
Extended Diestrus	High vs. controls	<0.001	–
Extended Metestrus	Overall	1	
Extended Metestrus	Low vs. controls	1	–
Extended Metestrus	Mid vs. controls	1	–
Extended Metestrus	High vs. controls	1	–
Extended Proestrus	Overall	1	
Extended Proestrus	Low vs. controls	1	–
Extended Proestrus	Mid vs. controls	1	–
Extended Proestrus	High vs. controls	1	–
Skipped Estrus	Overall	1	
Skipped Estrus	Low vs. controls	1	–
Skipped Estrus	Mid vs. controls	1	–
Skipped Estrus	High vs. controls	1	–
Skipped Diestrus	Overall	1	
Skipped Diestrus	Low vs. controls	1	–
Skipped Diestrus	Mid vs. controls	1	–
Skipped Diestrus	High vs. controls	1	–
Summary of Significant Groups			
Overall Tests	Mid vs. controls	<0.001	–
Overall Tests	High vs. controls	<0.001	–
Extended Estrus	Mid vs. controls	0.049	–
Extended Diestrus	Mid vs. controls	<0.001	–
Extended Diestrus	High vs. controls	<0.001	–

[a]Controls = Vehicle Control, Low = 156 mg/L, Mid = 312 mg/L, High = 625 mg/L.
[b]N means that the treated group had a lower probability of transitioning to the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the vehicle control group.

Table H-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	312 mg/L	625 mg/L	1,250 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	38.5 ± 1.3	39.9 ± 1.3	39.6 ± 1.1	37.9 ± 1.0
L. Cauda epididymis	0.0152 ± 0.0005	0.0151 ± 0.0004	0.0165 ± 0.0007	0.0289 ± 0.0134
L. Epididymis	0.0453 ± 0.0013	0.0433 ± 0.0009	0.0454 ± 0.0011	0.0443 ± 0.0010
L. Testis	0.1108 ± 0.0022	0.1102 ± 0.0021	0.1096 ± 0.0031	0.1100 ± 0.0026
Spermatid measurements				
Spermatid heads (10^6/testis)	19.73 ± 0.71	18.55 ± 0.72	19.71 ± 1.00	18.67 ± 0.95
Spermatid heads (10^6/g testis)	195.8 ± 6.1	187.1 ± 7.2	198.2 ± 8.6	189.4 ± 8.0
Epididymal spermatozoal measurements				
Sperm motility (%)	81.3 ± 0.7	81.9 ± 0.6	81.8 ± 0.9	80.3 ± 0.6
Sperm (10^6/cauda epididymis)	18.30 ± 1.20	17.25 ± 1.37	19.33 ± 2.42	15.70 ± 1.14
Sperm (10^6/g cauda epididymis)	1,231.8 ± 103.5	1,136.3 ± 72.2	1,161.6 ± 116.4	1,026.0 ± 58.9

[a]Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table H-5. Estrous Cycle Characterization for Female Mice in the Three-month Drinking Water Study of β-Picoline[a]

	0 mg/L	312 mg/L	625 mg/L	1,250 mg/L
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	27.2 ± 0.9	28.0 ± 1.1	27.6 ± 0.5	27.9 ± 0.9
Proportion of regular cycling females[b]	10/10	9/10	10/10	9/9
Estrous cycle length (days)	3.9 ± 0.13	4.1 ± 0.15[c]	4.2 ± 0.18	4.3 ± 0.62[c]
Estrous stages (% of cycle)				
Diestrus	35.8	33.3	35.8	43.3
Proestrus	0.0	0.0	0.0	0.0
Estrus	41.7	45.8	43.3	39.2
Metestrus	22.5	20.8	20.8	17.5

[a]Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices indicated no significant differences in the probability of altered cycle for any exposed group compared to the control group.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle was longer than 12 days or unclear in one of 10 animals.

Dose (mg/L)																					
0		E	D	D	D	D	D	D	D	D	D	D	D								
0								E	E	M	D	D	D	E	M	D	D	D	D		
0		D	D	D	D	D	D	D	D	D	D	D	D								
0								P	E	D	D	D	P	E	D	D	D	P	E		
0								P	E	D	D	D	P	E	D	D	D	P	E		
0		D	D	D	D	D	D	D	D	D	D	D	D								
0									D	E	M	D	D	E	M	D	D	E	E	D	
0		D	P	E	D	D	D	D	D	D	D	D	D								
0							D	P	E	D	D	D	P	E	D	D	D	D			
0									D	E	M	D	D	E	M	D	D	E	E	D	
156		E	D	D	D	D	D	D	D	D	D	D	D								
156								D	E	M	D	D	P	E	D	D	D	P	E		
156		D	D	E	D	D	D	D	D	D	D	D	D								
156					D	D	D	E	E	D	D	D	D	E	D	D					
156						D	D	P	E	M	D	D	P	E	D	D	D				
156								D	D	E	D	D	D	E	E	D	D	D	D		
156		D	D	D	P	E	D	D	D	D	D	D	D								
156				D	D	P	E	D	D	D	D	D	P	E	D						
156		D	D	E	D	D	D	D	D	D	D	D	D								
156					D	D	D	P	E	M	D	D	P	E	D	D					
312									E	D	D	D	P	E	D	D	D	P	E	D	
312						D	D	P	E	D	D	D	P	E	D	D	D				
312								D	D	E	M	D	D	E	E	D	D	D	D		
312									E	E	D	D	D	E	E	D	D	D	P	E	
312									E	D	D	D	P	E	D	D	D	D	D	D	
312								E	E	D	D	D	D	E	E	D	D	D	P		
312						D	D	P	E	D	D	D	P	E	D	D	D				
312					D	D	D	P	E	D	D	D	P	E	D	D					
312					D	D	D	E	E	D	D	D	P	E	D	D					
312								E	E	M	D	D	P	E	M	D	D	D	E		
625									E	D	D	D	P	E	D	D	D	P	E	D	
625					D	D	D	P	E	D	D	D	D	E	D	D					
625									E	D	D	D	D	E	E	D	D	D	P	E	
625							D	P	E	D	D	D	D	E	D	D	D	P			
625		D	D	D	D	D	D	D	D	D	D	D	D								
625								P	E	D	D	D	P	E	D	D	D	P	E		
625										E	M	D	D	E	E	D	D	D	D	E	D
625								P	E	D	D	D	P	E	D	D	D	E	E		
625								E	E	D	D	D	P	E	D	D	D	D	E		
625						D	D	P	E	D	D	D	D	E	D	D	D				

Figure H-1. Vaginal Cytology Plots for Female Rats in the Three-month Drinking Water Study of β-Picoline[a]

[a]D = diestrus, P = proestrus, E = estrus, M = metestrus.

Appendix I. Chemical Characterization and Dose Formulation Studies

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I.1. Procurement and Characterization

I.1.1. β-Picoline

β-picoline was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), in one lot (11108CI) that was used during the 3-month and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Chemistry Support Services (Columbus, OH) and the study laboratory at Battelle Columbus Operations (Columbus, OH). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). One additional lot (14517PS) obtained from Aldrich Chemical Company was used by the analytical chemistry laboratory for dose formulation stability studies and was not used in the 3-month or 2-year animal studies. Reports on analyses performed in support of the β-picoline studies are on file at the National Institute of Environmental Health Sciences.

Lot 11108CI, a clear, pale-yellow liquid, was identified as β-picoline by the analytical chemistry laboratory and the study laboratory using infrared (IR) spectroscopy; the analytical chemistry laboratory also used proton and carbon-13 nuclear magnetic resonance spectroscopy. All spectra were consistent with the literature spectra[63-65] of β-picoline and the structure of β-picoline. A representative IR spectrum is presented in Figure I-1.

Karl Fischer titration was used to determine the water content of lot 11108CI and elemental analyses were used to determine the carbon, hydrogen, and nitrogen content. The purity of lot 11108CI was determined by the analytical chemistry laboratory using gas chromatography (GC) by system A and differential scanning calorimetry (DSC). DSC was performed using a method which included a Perkin Elmer DSC-7 instrument (Perkin Elmer, Inc., Shelton, CT) scanning from −45° to −15°C (−45 to 10°C for the first replicate) with a temperature increase of 1°C per minute under a nitrogen atmosphere.

A) The GC system included a gas chromatograph (Agilent Inc., Palo Alto, CA) with flame ionization detection (FID), a 30 m × 0.25 mm, 0.5 µm film thickness column (Restek Corporation, Bellefonte, CA), an oven temperature program of 80°C, held for 4 minutes, increased at 5°C/minute to 130°C, held for 2 minutes, and helium carrier gas at a flow rate of 1.5 mL/minute.

Differential scanning calorimetry analysis indicated a purity of 96.4%. Karl Fischer titration indicated a 2.5% water content. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for β-picoline. GC with FID analysis indicated one major peak (β-picoline) and only two impurities with individual peak areas greater than 0.1%. The peak areas for the impurities represented a total of 1% of lot 11108CI (0.6% and 0.4%, respectively).

To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles sealed with Teflon®-lined lids under a nitrogen head space. Periodic reanalyses of the bulk chemical were performed by the study laboratory using GC by system A at the beginning and end of the 3-month and 2-year studies, and approximately every 6 months during the 2-year studies; no degradation of the bulk chemical occurred.

I.2. Preparation and Analysis of Dose Formulations

The dose formulations were prepared five times for the 3-month studies and approximately every 4 weeks for the 2-year studies. The dose formulations were prepared by mixing β-picoline with tap water (Table I-1). The pH was adjusted, if necessary, to bring it within the range of 6 to 7.5 by the addition of acetic acid, with stirring. The dose formulations were stored protected from light in sealed polypropylene carboys at 5°C for up to 29 (3-month studies) or 42 (2-year studies) days.

The dose formulations were determined to be true solutions; therefore, no homogeneity studies were performed. Stability studies of 10 µg/mL formulations were performed by the analytical chemistry laboratory using a high-performance liquid chromatography (HPLC) method that included an HPLC instrument (Waters, Corporation, Milford, MA), a Luna C18 (150 mm × 4.6 mm) column (Phenomenex, Torrance, CA), with ultraviolet (UV) light detection at 254 nm, a mobile phase of 5:94:1 (v:v:v) acetonitrile:5mM sodium pentane sulfonate:glacial acetic acid, isocratic, and a flow rate of 1 mL/minute. Stability was confirmed for at least 42 days for formulations stored in sealed polyethylene bottles protected from light at 5°C and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of β-picoline were performed by the study laboratory using HPLC/UV by a method similar to that described above. During the 3-month studies, the dose formulations were analyzed three times; all 15 dose formulations analyzed and used for rats and mice were within 10% of the target concentrations (Table I-2). Animal room samples of these dose formulations were also analyzed; all 15 animal room samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed at least every 12 weeks. All 50 dose formulations analyzed and used for rats and all 50 for mice were within 10% of the target concentrations (Table I-3). Animal room samples were also analyzed; all 12 animal room samples for rats and all 12 for mice were within 10% of the target concentrations.

Table I-1. Preparation and Storage of Dose Formulations in the Drinking Water Studies of β-Picoline

Three-month Studies	Two-year Studies
The 78 and 156 mg/L dose formulations were prepared by adding the appropriate amounts of β-picoline with rinsing by pipette or, for the 312, 625, and 1,250 mg/L dose formulations, by graduated cylinder, into approximately half the required amount of tap water in a 15-gallon Nalgene® calibrated tank, stirring with an overhead drum stirrer for approximately 15 minutes; then approximately 1 L of solution was dispensed through the tap into a beaker and poured back into the top of the tank with rinsing, then diluted to volume, and stirred an additional 5 minutes. The pH was determined and adjusted, if necessary, to bring it within the range of 6 to 7.5 by the addition of acetic acid, with stirring. The dose formulations were prepared five times.	Same as the 3-month studies through January 13, 2005, when the step requiring approximately 1 L of solution to be dispensed through the tap into a beaker and poured back into the top of the tank with rinsing was discontinued.
Chemical Lot Number	
11108CI	11108CI
Maximum Storage Time	
29 days	42 days
Storage Conditions	
Stored in sealed clear polypropylene carboys at 5°C, protected from light	Same as 3-month studies
Study Laboratory	
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

Table I-2. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Three-month Drinking Water Studies of β-Picoline

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
November 5, 2003	November 6, 2003	78	81.48	+4
		156	164.9	+6
		312	326.0	+4
		625	657.4	+5
		1,250	1,368	+9
December 3, 2003	December 5, 2003	78	75.84	−3
		156	156.3	0
		312	311.4	0
		625	623.9	0
		1,250	1,243	−1
January 20, 2004	January 23, 2004	78	79.93	+2
		156	157.8	+1
		312	309.9	−1
		625	673.9	+8
		1,250	1,302	+4
Animal Room Samples				
Rats				
November 5, 2003	December 5–6, 2003	78	75.23	−4
		156	154.0	−1
		312	304.3	−2
		625	618.0	−1
		1,250	1,222	−2
December 3, 2003	January 26–27, 2004	78	73.80	−5
		156	152.6	−2
		312	307.1	−2
		625	645.8	+3
		1,250	1,229	−2
January 20, 2004	March 12–13, 2004	78	75.20	−4
		156	150.1	−4
		312	299.4	−4
		625	641.4	+3
		1,250	1,200	−4

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Mice				
November 5, 2003	December 5–6, 2003	78	73.39	−6
		156	151.5	−3
		312	292.7	−6
		625	617.6	−1
		1,250	1,198	−4
December 3, 2003	January 26–27, 2004	78	79.30	+2
		156	158.6	+2
		312	311.2	0
		625	677.5	+8
		1,250	1,308	+5
January 20, 2004	March 12–13, 2004	78	74.65	−4
		156	149.6	−4
		312	303.4	−3
		625	633.5	+1
		1,250	1,214	−3

[a]Results of duplicate analyses.

Table I-3. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Two-year Drinking Water Studies of β-Picoline

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats				
October 26, 2004	October 28, 2004	156.25	154.5	−1
		312.5	320.0	+2
		312.5	327.4	+5
		625	640.3	+2
		625	639.3	+2
	December 7–9, 2004[b]	156.25	154.0	−2
		312.5	313.8	0
		625	621.8	−1
January 13, 2005	January 17, 2005	156.25	156.3	0
		312.5	318.8	+2
		312.5	330.1	+6
		625	646.6	+4
		625	637.6	+2
March 28, 2005	March 29–30, 2005	156.25	163.5	+5
		312.5	322.6	+3
		312.5	333.6	+7
		625	633.0	+1
		625	631.7	+1
June 14, 2005	June 15, 2005	156.25	156.1	0
		312.5	306.7	−2
		312.5	319.7	+2
		625	607.7	−3
		625	626.9	0
	July 28–29, 2005[b]	156.25	155.1	−1
		312.5	312.9	0
		625	626.9	0
September 1, 2005	September 9, 12–13, 2005	156.25	153.9	−2
		312.5	304.9	−2
		312.5	304.9	−2
		625	611.1	−2
		625	620.8	−1

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
November 15, 2005	November 16–17, 2005	156.25	147.9	−5
		312.5	317.8	+2
		312.5	306.0	−2
		625	622.8	0
		625	614.3	−2
February 2, 2006	February 7–8, 2006	156.25	156.6	0
		312.5	326.7	+5
		312.5	316.0	+1
		625	637.0	+2
		625	637.0	+2
	March 14–15, 2006[b]	156.25	151.9	−3
		312.5	321.2	+3
		625	625.3	0
April 20, 2006	April 21–22, 2006	156.25	155.7	0
		312.5	320.5	+3
		312.5	307.6	−2
		625	622.1	−1
		625	617.9	−1
July 6, 2006	July 11–12, 2006	156.25	154.7	−1
		312.5	322.0	+3
		312.5	312.5	0
		625	611.9	−2
		625	624.5	0
September 25, 2006	September 27, 2006	156.25	154.1	−1
		312.5	322.3	+3
		312.5	312.7	0
		625	613.4	−2
		625	629.5	+1
	November 14–15, 2006[b]	156.25	150.7	−4
		312.5	313.7	0
		625	599.6	−4

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Mice				
October 26, 2004	October 28, 2004	312.5	320.0	+2
		312.5	327.4	+5
		625	640.3	+2
		625	639.3	+2
		1,250	1,277	+2
	December 7–9, 2004[b]	312.5	309.6	−1
		625	629.9	+1
		1,250	1,243	−1
January 13, 2005	January 17, 2005	312.5	318.8	+2
		312.5	330.1	+6
		625	646.6	+4
		625	637.6	+2
		1,250	1,277	+2
March 28, 2005	March 29–30, 2005	312.5	322.6	+3
		312.5	333.6	+7
		625	633.0	+1
		625	631.7	+1
		1,250	1,277	+2
June 14, 2005	June 15, 2005	312.5	306.7	−2
		312.5	319.7	+2
		625	607.7	−3
		625	626.9	0
		1,250	1,237	−1
	July 28–29, 2005[b]	312.5	311.5	0
		625	597.5	−4
		1,250	1,199	−4
September 1, 2005	September 9, 12–13, 2005	312.5	304.9	−2
		312.5	304.9	−2
		625	611.1	−2
		625	620.8	−1
		1,250	1,214	−3

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
November 15, 2005	November 16–17, 2005	312.5	317.8	+2
		312.5	306.0	−2
		625	622.8	0
		625	614.3	−2
		1,250	1,231	−2
February 2, 2006	February 7–8, 2006	312.5	326.7	+5
		312.5	316.0	+1
		625	637.0	+2
		625	637.0	+2
		1,250	1,250	0
	March 14–15, 2006[b]	312.5	308.9	−1
		625	617.0	−1
		1,250	1,238	−1
April 20, 2006	April 21–22, 2006	312.5	320.5	+3
		312.5	307.6	−2
		625	622.1	−1
		625	617.9	−1
		1,250	1,205	−4
July 6, 2006	July 11–12, 2006	312.5	322.0	+3
		312.5	312.5	0
		625	611.9	−2
		625	624.5	0
		1,250	1,237	−1
September 25, 2006	September 27, 2006	312.5	322.3	+3
		312.5	312.7	0
		625	613.4	−2
		625	629.5	+1
		1,250	1,266	+1
	November 14–15, 2006[b]	312.5	305.5	−2
		625	619.6	−1
		1,250	1,226	−2

[a]Results of duplicate analyses.
[b]Animal room samples.

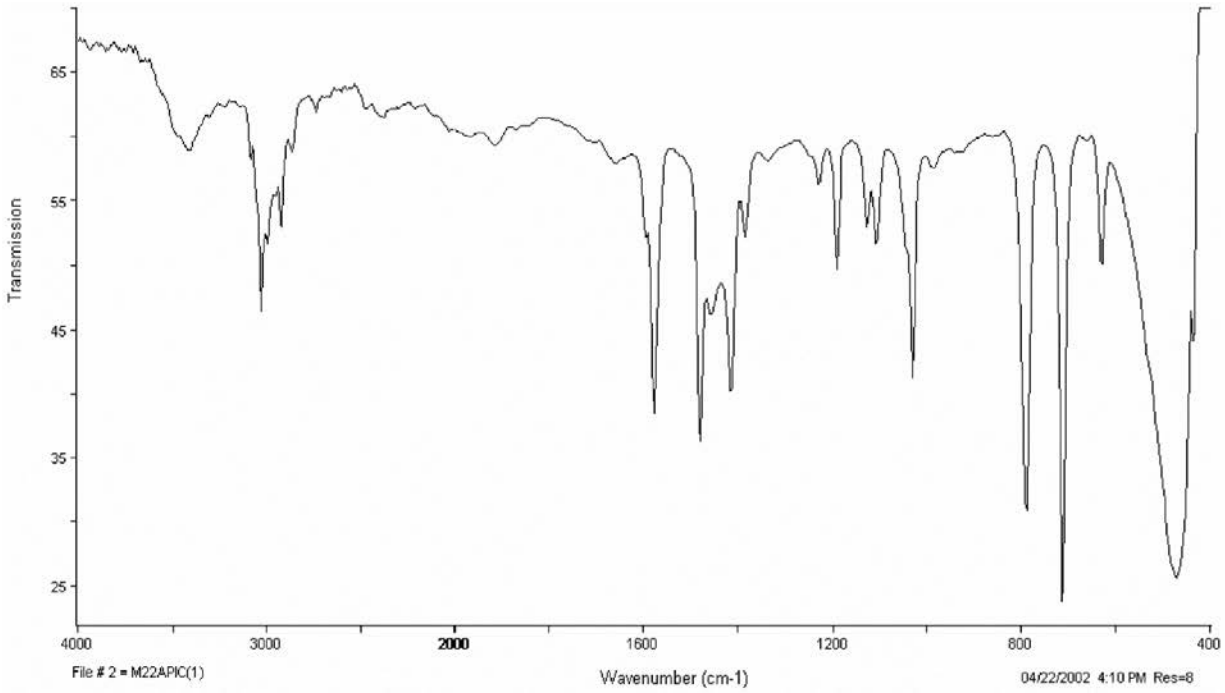

Figure I-1. Infrared Absorption Spectrum of β-Picoline

Appendix J. Water and Compound Consumption in the Two-year Drinking Water Studies of β-Picoline

Tables

Table J-1. Water and Compound Consumption by Male Rats in the Two-year Drinking Water Study of β-Picoline

Week	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	14.8	116	14.7	115	20	14.3	116	38	11.3	115	62
2	16.3	150	15.9	147	17	15.9	148	34	12.9	142	57
3	17.5	181	17.0	179	15	16.7	181	29	13.5	171	49
4	17.8	212	17.3	208	13	17.2	208	26	14.1	195	45
5	16.9	232	17.1	229	12	16.6	230	23	14.0	215	41
6	16.2	248	16.3	248	10	16.3	248	21	13.8	234	37
7	15.6	262	15.3	263	9	14.9	263	18	13.4	247	34
8	15.5	277	15.7	276	9	14.8	278	17	12.7	259	31
9	15.3	289	15.6	289	8	14.4	290	16	12.5	271	29
10	14.9	301	15.2	301	8	14.5	302	15	12.4	281	28
11	15.1	311	15.5	311	8	14.8	313	15	12.6	289	27
12	15.5	322	15.4	321	8	14.7	323	14	12.6	298	26
13	15.8	330	15.9	329	8	15.3	330	15	13.0	305	27
17	15.2	357	14.8	354	7	14.9	357	13	12.0	332	23
21	15.7	378	16.0	376	7	15.7	383	13	13.6	352	24
25	16.0	400	15.9	399	6	15.8	402	12	13.8	374	23
29	14.7	409	14.8	409	6	14.9	416	11	13.0	387	21
33	15.4	423	15.0	420	6	14.7	429	11	13.0	400	20
37	15.9	434	15.4	435	6	14.9	441	11	12.9	410	20
41	15.0	442	14.4	441	5	14.7	453	10	13.0	422	19
45	15.1	453	14.7	453	5	14.9	462	10	12.9	431	19
49	16.3	458	16.0	460	5	15.6	467	10	13.7	436	20
53	16.3	468	16.2	467	5	16.0	477	11	14.5	445	20
57	16.9	478	16.6	478	5	16.1	487	10	14.2	452	20
61	17.0	481	16.8	480	6	15.7	487	10	13.8	453	19
65	16.4	486	16.2	487	5	15.8	493	10	13.6	457	19
69	16.5	493	15.9	490	5	15.3	499	10	13.8	463	19
73	17.2	501	16.8	499	5	15.9	506	10	13.9	471	19
77	16.5	504	16.3	506	5	15.2	510	9	13.5	472	18
81	16.5	508	15.8	508	5	14.9	511	9	14.1	475	19
85	17.7	512	16.7	514	5	15.6	514	10	13.7	474	18
89	16.3	516	15.4	516	5	13.6	516	8	12.6	474	17
93	16.2	512	15.6	508	5	13.7	504	9	12.9	470	17
97	16.7	507	16.6	506	5	14.9	502	9	12.7	459	17
101	19.0	503	17.3	497	5	15.8	501	10	13.3	451	18

Week	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
Mean for Weeks											
1–13	15.9	249	15.9	247	11	15.4	248	22	13.0	232	38
14–52	15.5	417	15.2	416	6	15.1	423	11	13.1	394	21
53–101	16.9	498	16.3	497	5	15.3	501	10	13.6	463	18

[a]Grams of water consumed per animal per day.
[b]Milligrams of β-picoline consumed per kilogram body weight per day.

Table J-2. Water and Compound Consumption by Female Rats in the Two-year Drinking Water Study of β-Picoline

Week	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	11.0	99	10.6	99	17	10.4	100	33	8.7	100	54
2	12.1	117	11.9	116	16	10.9	116	29	8.7	114	48
3	12.4	132	12.4	132	15	11.8	131	28	9.3	124	47
4	11.6	144	11.6	144	13	11.0	144	24	9.4	137	43
5	11.1	152	11.3	153	12	10.8	154	22	9.5	145	41
6	11.1	162	11.6	165	11	10.4	165	20	9.5	157	38
7	10.6	170	11.1	172	10	10.7	171	20	8.8	163	34
8	10.8	175	10.7	176	10	10.0	175	18	8.6	168	32
9	10.2	180	10.3	181	9	9.8	181	17	8.4	172	31
10	10.4	184	10.0	186	8	9.3	185	16	8.4	175	30
11	10.3	188	10.7	191	9	9.5	188	16	8.8	180	31
12	10.4	192	10.5	193	9	9.5	192	16	8.8	183	30
13	10.5	195	10.4	196	8	8.9	194	14	8.6	185	29
17	9.7	207	10.4	208	8	9.8	208	15	8.5	197	27
21	10.4	218	10.3	218	7	9.9	216	14	8.6	203	26
25	10.9	223	10.9	226	8	10.3	225	14	9.6	213	28
29	10.5	233	10.1	233	7	9.1	228	13	8.7	220	25
33	10.8	238	10.4	241	7	10.1	238	13	8.9	229	24
37	10.1	247	9.7	248	6	9.4	245	12	8.5	235	23
41	10.8	253	9.9	256	6	9.8	251	12	8.6	242	22
45	10.8	264	10.3	265	6	9.5	261	11	9.1	251	23
49	11.6	270	10.7	271	6	10.0	265	12	9.7	256	24
53	12.1	282	11.0	281	6	10.8	275	12	9.5	265	22
57	12.1	294	11.4	293	6	11.0	285	12	10.3	274	24
61	12.5	302	11.2	299	6	11.1	289	12	10.3	278	23
65	12.7	311	11.7	309	6	11.6	299	12	10.4	286	23
69	12.9	319	11.9	319	6	11.2	307	11	10.8	294	23
73	13.2	328	12.6	329	6	11.9	319	12	11.7	305	24
77	13.3	340	12.1	336	6	11.6	326	11	11.0	311	22
81	13.4	344	12.5	340	6	11.6	331	11	11.5	313	23
85	13.3	348	13.6	346	6	12.8	336	12	11.9	315	24
89	13.0	352	12.8	353	6	12.0	344	11	11.3	322	22
93	16.2	353	16.9	351	8	15.3	348	14	13.0	322	25
97	13.8	350	15.1	355	7	12.5	348	11	12.1	326	23
101	14.2	339	15.7	349	7	13.9	346	13	13.9	323	27

Week	0 mg/L		156.25 mg/L			312.5 mg/L			625 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
Mean for Weeks											
1–13	11.0	161	11.0	162	11	10.2	161	21	8.9	154	38
14–52	10.6	239	10.3	241	7	9.8	237	13	8.9	227	25
53–101	13.3	328	13.0	328	6	12.1	319	12	11.4	303	23

[a]Grams of water consumed per animal per day.
[b]Milligrams of β-picoline consumed per kilogram body weight per day.

Table J-3. Water and Compound Consumption by Male Mice in the Two-year Drinking Water Study of β-Picoline

Week	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	3.9	23.7	3.7	23.8	49	3.7	23.6	98	3.4	23.7	180
2	3.8	25.8	3.4	25.5	42	3.5	25.2	87	3.3	25.2	164
3	3.8	27.2	3.6	27.0	42	3.4	26.5	80	3.3	26.6	155
4	3.7	28.8	3.7	28.7	40	3.5	28.1	78	3.6	28.1	160
5	3.8	30.9	3.6	30.5	37	3.5	29.8	73	3.5	29.9	147
6	3.8	33.0	3.7	32.5	36	3.6	31.6	71	3.4	31.5	135
7	4.0	35.0	3.8	34.5	35	3.7	33.3	70	3.7	33.3	139
8	3.6	36.6	3.6	35.9	31	3.5	34.8	63	3.5	34.7	126
9	3.7	38.1	3.4	37.5	28	3.4	36.2	59	3.2	36.2	111
10	3.7	39.7	3.5	39.0	28	3.5	37.7	58	3.3	37.7	110
11	3.5	41.2	3.4	40.7	26	3.4	39.3	54	3.2	39.3	102
12	3.6	42.9	3.5	42.2	26	3.3	40.8	51	3.2	40.8	98
13	3.6	44.1	3.4	43.2	25	3.4	41.8	51	3.2	41.8	96
17	3.6	48.0	3.5	46.8	23	3.2	45.4	44	3.2	45.3	88
21	3.7	49.5	3.4	48.5	22	3.4	47.2	45	3.4	46.5	91
25	3.6	50.2	3.4	49.1	22	3.3	47.9	43	3.1	47.1	82
29	4.2	51.9	3.8	50.8	23	3.5	49.6	44	3.4	48.5	88
33	4.1	52.9	3.6	52.0	22	3.4	50.5	42	3.0	49.1	76
37	4.2	54.0	3.8	52.9	22	3.6	51.3	44	3.2	49.7	81
41	4.2	55.1	3.9	54.0	23	3.5	52.2	42	3.0	50.3	75
45	4.4	55.9	4.1	55.3	23	3.7	53.2	44	3.0	51.0	74
49	4.5	56.7	4.2	55.7	24	3.7	53.2	43	3.2	51.5	78
53	4.8	56.7	4.4	55.9	25	4.0	54.1	46	3.3	52.1	79
57	5.0	57.1	4.6	56.5	25	4.1	53.9	48	3.4	51.9	82
61	4.9	57.0	4.5	56.0	25	4.2	53.4	49	3.3	51.1	81
65	4.8	56.3	4.6	55.6	26	4.0	52.9	47	3.1	50.8	76
69	4.8	56.3	4.6	55.6	26	4.1	53.1	48	3.2	49.9	80
73	4.9	56.2	4.6	55.0	26	4.4	52.9	52	3.4	48.8	87
77	4.7	55.4	4.6	54.1	27	4.1	52.5	49	3.1	47.2	82
81	4.9	54.3	4.8	52.5	29	4.4	51.4	54	3.3	46.1	89
85	4.5	53.2	4.3	51.4	26	3.9	50.3	48	3.3	44.9	92
89	4.5	50.3	4.1	51.5	25	3.8	49.0	48	3.2	42.7	94
93	4.4	48.5	4.2	51.1	26	3.8	46.5	51	3.2	40.6	99
97	4.4	46.8	4.3	48.6	28	3.8	45.0	53	3.3	37.9	109
101	4.4	43.0	4.4	47.6	29	4.0	42.7	59	3.4	36.8	115

Week	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
Mean for Weeks											
1–13	3.7	34.4	3.6	33.9	34	3.5	33.0	69	3.4	33.0	132
14–52	4.1	52.7	3.7	51.7	23	3.5	50.1	43	3.2	48.8	81
53–101	4.7	53.2	4.5	53.2	26	4.0	50.6	50	3.3	46.2	90

[a]Grams of water consumed per animal per day.
[b]Milligrams of β-picoline consumed per kilogram body weight per day.

Table J-4. Water and Compound Consumption by Female Mice in the Two-year Drinking Water Study of β-Picoline

Week	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	2.5	18.6	2.4	18.8	40	2.3	18.7	77	2.1	18.5	142
2	2.5	19.7	2.4	19.7	38	2.3	19.2	75	2.0	19.1	131
3	2.6	20.7	2.3	20.6	35	2.4	20.2	74	2.2	20.0	137
4	2.8	21.4	2.6	21.7	37	2.4	21.3	70	2.3	20.8	138
5	2.7	22.3	2.7	22.7	37	2.4	22.1	68	2.2	21.5	128
6	2.8	23.4	2.6	23.4	35	2.5	22.7	69	2.3	22.4	128
7	3.0	24.5	2.9	24.6	37	2.7	23.7	71	2.5	23.3	134
8	2.8	25.1	2.8	25.0	35	2.6	24.4	67	2.3	23.6	122
9	2.8	26.6	2.7	26.6	32	2.5	25.5	61	2.3	24.8	116
10	2.9	27.5	2.7	27.7	30	2.7	27.1	62	2.3	25.8	112
11	2.8	29.3	2.7	29.3	29	2.6	28.5	57	2.2	26.5	104
12	2.8	30.5	2.7	30.6	28	2.6	30.1	54	2.4	27.9	108
13	2.8	31.8	2.8	32.1	27	2.7	31.1	54	2.3	28.9	99
17	2.7	37.6	2.7	37.5	23	2.6	36.5	45	2.4	33.7	89
21	2.6	43.3	2.5	42.9	18	2.6	41.2	39	2.3	38.8	74
25	2.3	47.3	2.3	47.2	15	2.2	45.0	31	1.9	42.3	56
29	2.3	50.4	2.3	50.2	14	2.2	47.8	29	2.1	45.3	58
33	2.5	53.3	2.4	53.3	14	2.4	51.0	29	2.0	48.2	52
37	2.5	55.2	2.5	55.6	14	2.4	52.9	28	2.2	50.3	55
41	2.5	57.5	2.5	57.3	14	2.4	54.8	27	2.2	51.9	53
45	2.5	58.6	2.5	58.4	13	2.3	55.4	26	2.2	53.0	52
49	2.6	59.7	2.7	59.5	14	2.6	57.1	28	2.4	54.7	55
53	2.8	61.5	2.8	60.5	15	3.0	58.5	32	2.6	55.5	59
57	2.7	62.8	2.8	62.6	14	3.0	60.2	31	2.5	56.8	55
61	2.9	63.8	3.3	63.6	16	3.2	61.3	33	2.6	57.5	57
65	3.0	65.0	3.0	64.8	15	3.2	62.2	32	2.5	58.1	54
69	2.9	65.6	3.1	65.4	15	3.1	62.5	31	2.6	59.0	55
73	3.4	67.3	3.3	66.1	16	3.4	63.4	34	2.9	59.3	61
77	3.2	67.4	3.3	64.7	16	3.1	63.1	31	2.6	59.7	54
81	3.6	66.2	3.9	65.1	19	3.2	64.1	31	2.9	60.0	60
85	3.5	65.3	3.7	65.2	18	3.2	64.0	31	2.8	60.3	58
89	3.5	64.9	3.3	64.8	16	3.2	63.7	31	2.6	58.6	56
93	4.0	63.9	3.8	63.6	19	3.6	62.5	36	2.9	56.5	64
97	4.1	61.2	3.8	61.0	20	3.9	60.2	41	3.0	56.5	66
101	3.7	60.6	3.8	57.4	21	4.0	57.5	44	3.5	53.3	82

Week	0 mg/L		312.5 mg/L			625 mg/L			1,250 mg/L		
	Water[a] (g/day)	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose[b] (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
Mean for Weeks											
1–13	2.8	24.7	2.6	24.8	34	2.5	24.2	66	2.3	23.3	123
14–52	2.5	51.4	2.5	51.3	15	2.4	49.1	31	2.2	46.5	60
53–101	3.3	64.3	3.4	63.4	17	3.3	61.8	34	2.8	57.8	60

[a]Grams of water consumed per animal per day.
[b]Milligrams of β-picoline consumed per kilogram body weight per day.

Appendix K. Ingredients, Nutrient Composition, and Contaminant Levels in NTP-2000 Rat and Mouse Ration

Tables

Table K-1. Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a]Wheat middlings as carrier.
[b]Calcium carbonate as carrier.

Table K-2. Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	–
Niacin	23 mg	–
Folic acid	1.1 mg	–
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	–
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	–
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a]Per kg of finished product.

Table K-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.6 ± 0.68	13.5–16.3	25
Crude fat (% by weight)	8.2 ± 0.35	7.6–9.3	25
Crude fiber (% by weight)	9.2 ± 0.46	8.4–10.0	25
Ash (% by weight)	4.9 ± 0.25	4.6–5.4	25
Amino Acids (% of total diet)			
Arginine	0.778 ± 0.068	0.670–0.970	21
Cystine	0.220 ± 0.025	0.150–0.250	21
Glycine	0.701 ± 0.042	0.620–0.800	21
Histidine	0.354 ± 0.079	0.270–0.680	21
Isoleucine	0.544 ± 0.045	0.430–0.660	21
Leucine	1.092 ± 0.068	0.960–1.240	21
Lysine	0.704 ± 0.112	0.310–0.840	21
Methionine	0.409 ± 0.047	0.260–0.490	21
Phenylalanine	0.626 ± 0.040	0.540–0.720	21
Threonine	0.503 ± 0.043	0.430–0.610	21
Tryptophan	0.148 ± 0.027	0.110–0.200	21
Tyrosine	0.397 ± 0.058	0.280–0.540	21
Valine	0.666 ± 0.044	0.550–0.730	21
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.227	3.49–4.54	21
Linolenic	0.30 ± 0.030	0.21–0.35	21
Vitamins			
Vitamin A (IU/kg)	3,940 ± 775	2,340–5,590	25
Vitamin D (IU/kg)	1,000[a]	–	–
α-Tocopherol (ppm)	80.1 ± 22.48	27.0–124.0	21
Thiamine (ppm)[b]	7.8 ± 1.20	6.3–10.5	25
Riboflavin (ppm)	7.1 ± 1.91	4.20–11.20	21
Niacin (ppm)	78.6 ± 9.16	66.4–98.2	21
Pantothenic acid (ppm)	27.1 ± 12.89	17.4–81.0	21
Pyridoxine (ppm)[b]	9.47 ± 2.01	6.4–13.7	21
Folic acid (ppm)	1.63 ± 0.49	1.15–3.27	21
Biotin (ppm)	0.319 ± 0.10	0.200–0.704	21
Vitamin B_{12} (ppb)	53.8 ± 40.6	18.3–174.0	21
Choline (ppm)[b]	2,885 ± 459	1,820–3,790	21

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.977 ± 0.048	0.895–1.080	25
Phosphorus (%)	0.567 ± 0.033	0.499–0.623	25
Potassium (%)	0.663 ± 0.027	0.626–0.732	21
Chloride (%)	0.387 ± 0.039	0.300–0.474	21
Sodium (%)	0.190 ± 0.016	0.160–0.222	21
Magnesium (%)	0.216 ± 0.063	0.185–0.490	21
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	185 ± 40.1	135–311	21
Manganese (ppm)	51.6 ± 10.49	21.0–73.1	21
Zinc (ppm)	53.6 ± 8.62	43.3–78.5	21
Copper (ppm)	7.07 ± 2.611	3.21–16.30	21
Iodine (ppm)	0.497 ± 0.209	0.158–0.972	21
Chromium (ppm)	0.684 ± 0.279	0.330–1.380	20
Cobalt (ppm)	0.26 ± 0.164	0.11–0.86	19

[a]From formulation.
[b]As hydrochloride (thiamine and pyridoxine) or chloride (choline).

Table K-4. Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.23 ± 0.058	0.16–0.39	25
Cadmium (ppm)	0.05 ± 0.007	0.04–0.07	25
Lead (ppm)	0.09 ± 0.014	0.07–0.13	25
Mercury (ppm)	<0.02	–	25
Selenium (ppm)	0.29 ± 0.099	0.18–0.49	25
Aflatoxins (ppb)	<5.00	–	25
Nitrate nitrogen (ppm)[c]	13.78 ± 7.33	4.76–36.8	25
Nitrite nitrogen (ppm)[c]	<0.61	–	25
BHA (ppm)[d]	<1.0	–	25
BHT (ppm)[d]	<1.0	–	25
Aerobic plate count (CFU/g)	10 ± 0.0	10.0–10.0	25
Coliform (MPN/g)	3.0 ± 0.0	3.0–3.0	25
Escherichia coli (MPN/g)	<10	–	25
Salmonella (MPN/g)	Negative	–	25
Total nitrosoamines (ppb)[e]	4.95 ± 1.68	2.0–9.9	25
N-Nitrosodimethylamine (ppb)[e]	2.8 ± 1.21	1.0–6.3	25
N-Nitrosopyrrolidine (ppb)[e]	2.1 ± 0.73	1.1–3.6	25
Pesticides (ppm)			
α-BHC	<0.01	–	25
β-BHC	<0.02	–	25
γ-BHC	<0.01	–	25
δ-BHC	<0.01	–	25
Heptachlor	<0.01	–	25
Aldrin	<0.01	–	25
Heptachlor epoxide	<0.01	–	25
DDE	<0.01	–	25
DDD	<0.01	–	25
DDT	<0.01	–	25
HCB	<0.01	–	25
Mirex	<0.01	–	25
Methoxychlor	<0.05	–	25
Dieldrin	<0.01	–	25
Endrin	<0.01	–	25

	Mean ± Standard Deviation[b]	Range	Number of Samples
Telodrin	<0.01	–	25
Chlordane	<0.05	–	25
Toxaphene	<0.10	–	25
Estimated PCBs	<0.20	–	25
Ronnel	<0.01	–	25
Ethion	<0.02	–	25
Trithion	<0.05	–	25
Diazinon	<0.10	–	25
Methyl chlorpyrifos	0.138 ± 0.128	0.020–0.416	25
Methyl parathion	<0.02	–	25
Ethyl parathion	<0.02	–	25
Malathion	0.245 ± 0.243	0.020–0.997	25
Endosulfan I	<0.01	–	25
Endosulfan II	<0.01	–	25
Endosulfan sulfate	<0.03	–	25

[a]All samples were irradiated. CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride.
[b]For values less than the limit of detection, the detection limit is given as the mean.
[c]Sources of contamination: alfalfa, grains, and fish meal.
[d]Sources of contamination: soy oil and fish meal.
[e]All values were corrected for percent recovery.

Appendix L. Sentinel Animal Program

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Tables

L.1. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via sera or feces from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

For the 3-month studies, serum samples were collected from five male and five female sentinel rats and mice at 1 month and at study termination. For the 2-year studies, serum samples were collected from five male and five female extra rats and mice at 1 month, from five male and five female sentinel rats and mice at 6, 12, and 18 months, and from five male and five female 625 mg/L rats and 1,250 mg/L mice at study termination. Fecal samples were taken from five male and five female sentinel mice at 18 months in the 2-year study for *Helicobacter spp.* by polymerase chain reaction testing. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Table L-1. Laboratory Methods and Agents Tested for in the Sentinel Animal Program

Method and Test	Time of Collection
Rats	
Three-month Study	
ELISA	
PVM (pneumonia virus of mice)	Study start, 1 month, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
Sendai	Study start
Two-year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
RCV/SDA	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1, 6, 12, and 18 months, study termination
Mice	
Three-month Study	
ELISA	

Method and Test	Time of Collection
Ectromelia virus	Study start, 1 month, study termination
EDIM (epizootic diarrhea of infant mice)	Study start, 1 month, study termination
GDVII (mouse encephalomyelitis virus)	Study start, 1 month, study termination
LCM (lymphocytic choriomeningitis virus)	Study start, 1 month, study termination
Mouse adenoma virus	Study start, 1 month, study termination
MHV (mouse hepatitis virus)	Study start, 1 month, study termination
PVM	Study start, 1 month, study termination
Reovirus 3	Study start, 1 month, study termination
Sendai	Study start, 1 month, study termination
Immunofluorescence Assay	
Parvovirus	Study start, 1 month, study termination
Two-year Study	
ELISA	
Ectromelia virus	Study start, 1, 6, 12, and 18 months, study termination
EDIM	Study start, 1, 6, 12, and 18 months, study termination
GDVII	Study start, 1, 6, 12, and 18 months, study termination
LCM	Study start, 1, 6, 12, and 18 months, study termination
MMV, VP2 (mouse minute virus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
MPV, VP2 (mouse parvovirus, viral protein 2)	Study start, 1, 6, 12, and 18 months, study termination
Mouse adenoma virus-1	12, 18 months, study termination
Mouse adenoma virus-FL	Study start, 1, 6 months
MHV	Study start, 1, 6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	Study start, 1, 6, 12, and 18 months, study termination
Reovirus 3	Study start, 1, 6, 12, and 18 months, study termination
Sendai	Study start, 1, 6, 12, and 18 months, study termination
Immunofluorescence Assay	
GDVII	12 months
MPV, VP2	18 months
Mouse adenoma virus-1	1 month
MCMV (mouse cytomegalovirus)	Study termination
Reovirus 3	1, 6 months
Polymerase Chain Reaction	
Helicobacter species	18 months

L.2. Results

All test results were negative.

Appendix M. Summary of Peer Review Panel Comments

On February 8, 2012, the draft Technical Report on the toxicology and carcinogenesis studies of β-picoline received public review by the National Toxicology Program's Technical Reports Peer Review Panel. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. M.E. Wyde, NIEHS, introduced the toxicology and carcinogenesis studies of β-picoline by describing the uses of the chemical and its structural similarity to pyridine. Much of the initial knowledge used to design the studies was derived from data on pyridine. Dr. Wyde described the design and results of the studies in rats and mice, including nonneoplastic and neoplastic lesions in test animals. The proposed conclusions were *no evidence of carcinogenic activity* of β-picoline in male F344/N rats, *some evidence of carcinogenic activity* of β-picoline in female F344/N rats, *equivocal evidence of carcinogenic activity* of β-picoline in male B6C3F1/N mice, and *clear evidence of carcinogenic activity* of β-picoline in female B6C3F1/N mice.

Dr. Mirsalis, the first primary reviewer, felt that it was a good report and he agreed with the proposed conclusions. He noted that the purity of the test article was 96%, and inquired about the other 4%. He requested more discussion on the compound's palatability. He noted the "high" 78% incidence of hepatocellular adenoma in the female mouse controls and questioned the significance of the increase in the incidences of hepatocellular adenoma in the exposed groups. Finally, he suggested that β-picoline might be a good candidate for reproductive toxicity tests in rats.

Dr. Pino, the second primary reviewer, recommended that the conclusion for male F344/N rats should have been *equivocal evidence of carcinogenic activity* based on the alveolar/bronchiolar carcinomas, as the report indicated that while the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in males were similar between control and exposed groups, these observations (referring to the carcinomas in males) may suggest a treatment related progression from benign tumors to malignancy. He asked for more discussion in the text about whether or not hepatocellular adenomas in the female mice might be compound related, which was not mentioned in the conclusion. He asked whether the incidence of multiple alveolar/bronchiolar carcinoma in male mice was above the historical range, because if so, he felt that it should be included in the conclusion. He asked why an increased severity of nephropathy was seen in the 3-month study in male rats, but not in the 2-year study at the same doses. He asked whether the estrous data in rats were skewed by the fact that the estrous cycle was longer than 12 days or unclear in four of the 10 control rats, because if so, he felt that information should be indicated in the discussion.

Dr. Alcorn, the third primary reviewer, questioned the exposure concentrations selected for the studies. She believed that they may have been too high given changes in body weight and water consumption in the 3-month studies. Based on those concerns, she endorsed changing the conclusion for female rats from *some evidence* to *equivocal evidence*. She also noted that the control mice lost significant weight in the last year of the study. She asked why the CYP2B1 liver microsomes were assessed, as there was no indication of the importance in the report. She said she would like to see less reference to pyridine in the report.

Dr. Wyde said that most of the impurity in the test article was water, and that there were two impurities at 0.6% and 0.4%. Regarding the high incidence of liver neoplasms in the female mouse controls, he said the proposed conclusion was mainly based on the hepatoblastomas and the hepatocellular carcinomas rather than the adenomas. He said the NTP would consider conducting reproductive studies. He discussed the rationale for the no evidence call in male rats. Dr. S.A. Elmore, NIEHS, explained the approach for evaluating the oral carcinomas. Dr. Mirsalis recommended including information about the compound's purity in the report.

Dr. Wyde said the intent of the exposure concentration selection had been to be sure the animals were challenged sufficiently. He said the information on pyridine was used as a starting point in the study design due to the lack of information on β-picoline. He said that the body weight loss in mice in the second year of the study was a typical response. He also explained that the call of *some evidence of carcinogenic activity* in the female rats was based on the occurrence of lung neoplasms in all three exposed groups, increased rates of hyperplasia in the exposed groups, and the potential for the adenomas to progress to carcinomas.

Dr. Roberts noted that there were at least two proposed changes to the conclusions. Dr. Pino said that perhaps the call for male rats should be *equivocal evidence of carcinogenic activity* based on the alveolar/bronchiolar carcinomas, or that the sentence regarding a possible compound-related progression to malignancy be deleted.

Dr. Anderson, private consultant, said there may have been some confusion as to nomenclature for reviewers who were not accustomed to reading the technical reports. Dr. M.J. Hooth, NIEHS, explained that the NTP makes calls assigning one level of evidence for each sex and species based upon the highest call for each. Dr. Alcorn said she accepted Dr. Wyde's explanation about dosing and was comfortable with the proposed conclusion regarding the female rats. Dr. Roberts called for a motion on the conclusions for β-picoline. Dr. Mirsalis moved to accept the conclusions as written and Dr. Alcorn seconded the motion. The panel accepted unanimously with 10 votes in favor to accept the conclusions as written.

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ISSN 2378-8925