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NTP Technical Report on the Toxicity Studies of

o-Chloropyridine (CASRN 109-09-1) Administered Dermally and in Drinking Water to F344/N Rats and B6C3F1/N Mice

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NTP Technical Report on the Toxicity Studies of *o*-Chloropyridine (CASRN 109-09-1) Administered Dermally and in Drinking Water to F344/N Rats and B6C3F1/N Mice

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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 Toxicity Study Report series began in 1991. The studies described in the Toxicity Study Report series are designed and conducted to characterize and evaluate the toxicologic potential of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in the Toxicity Study 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 toxic 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.

NTP Toxicity Study Reports are indexed in the National Center for Biotechnology Information (NCBI) Bookshelf and are available free of charge electronically on the NTP website (http://ntp.niehs.nih.gov). Additional information regarding this study may be requested through Central Data Management (CDM) at cdm@niehs.nih.gov. Toxicity data are available through NTP's Chemical Effects in Biological Systems (CEBS) database.

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This report has been reformatted to meet new NTP publishing requirements; its content has not changed.

About This Report

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Peer Review

The draft *NTP Technical Report on the Toxicity Studies of o-Chloropyridine (CASRN 109-09-1) Administered Dermally and in Drinking Water to F344/N Rats and B6C3F1/N Mice* was evaluated by the reviewers listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determined if the design and conditions of these NTP studies were appropriate and ensured that this NTP Toxicity Study Report presented the experimental results and conclusions fully and clearly.

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Abstract

o-Chloropyridine is used as an intermediate in synthetic organic, pharmaceutical, and agricultural chemical (fungicides, herbicides) manufacture. It is also used as a catalyst for phase transfer and is a key intermediate in the manufacture of pyrithione-based biocides for use in cosmetics and various pharmaceutical products. *o*-Chloropyridine is available in purified (99%), technical (95%), or crude (80%) grades. *o*-Chloropyridine was nominated for testing by NTP based on increasing production and use as a site-limited pharmaceutical and agrochemical intermediate, the potential for occupational and environmental exposures during its manufacture, its persistence in the environment (lasting longer than 6 months), evidence of mutagenicity based on results of several short-term test systems, and suspicion of carcinogenicity based on effects associated with structurally related chemicals. Male and female F344/N rats and B6C3F1/N mice received *o*-chloropyridine (99% pure) dermally for 2 weeks or in drinking water for 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse peripheral blood erythrocytes.

In the 2-week dermal studies, groups of five male and five female rats and mice were administered *o*-chloropyridine in ethanol 5 days per week over a 16-day period (12 dose days) at doses of 0, 6.25, 12.5, 25, 50, or 100 mg *o*-chloropyridine/kg body weight. Vehicle control animals were administered ethanol alone. A constant concentration of test chemical per dose concentration was administered to each animal at volumes of 0.5 mL/kg body weight for rats and 2 mL/kg for mice. All dosed rats and mice survived to the end of the studies. The mean body weights of all dosed groups of rats and mice were similar to those of the vehicle control groups. Liver weights of 50 and 100 mg/kg male rats were significantly greater than those of the vehicle controls. No gross or microscopic lesions were considered related to *o*-chloropyridine administration.

In the 3-month toxicity studies, groups of 10 male and 10 female F344/N rats and B6C3F1/N mice were exposed to *o*-chloropyridine in drinking water at concentrations of 0, 10, 30, 100, 300, or 1,000 ppm (equal to average daily doses of approximately 1, 3, 9, 25, and 65 mg *o*-chloropyridine/kg body weight to male rats, 1, 3, 9, 27, and 70 mg/kg to female rats, 1.5, 4.5, 15, 41, and 110 mg/kg to male mice, and 1.3, 4, 12, 38, and 92 mg/kg to female mice) for 3 months. Additional groups of 10 male and 10 female rats designated for clinical pathology testing were exposed to the same concentrations for 22 days.

All rats survived to the end of the study. Mean body weights of male and female rats exposed to 1,000 ppm were significantly less than those of the controls. Water consumption by the 1,000 ppm rats was less than that by the control groups during the first week of the study. In the 1,000 ppm groups, thinness was noted in seven of 10 male rats and all female rats on day 8, likely due to dehydration, and also in five of 10 male rats on day 64. The absolute and relative (except 100 ppm) right kidney weights of all exposed groups of male rats and of groups of female rats (≥30 ppm) were greater than controls. Absolute and relative liver weights of male rats (≥100 ppm) and female rats (≥30 ppm) were significantly greater than those of the control groups. Epididymal sperm counts were significantly lower in male rats exposed to 1,000 ppm, indicating that *o*-chloropyridine exhibits the potential to be a reproductive toxicant. In the liver of male rats, the incidence of clear cell focus (1,000 ppm) and the incidences and severities of hepatocyte cytoplasmic vacuolization (≥300 ppm) were significantly higher. The incidence of hepatocyte cytoplasmic vacuolization was significantly greater in female rats also (1,000 ppm).

There were also several low-magnitude histologic and hematologic responses in male and female rats suggesting an erythron effect characterized by a decreased erythron (≥300 ppm males and females) with a compensatory erythropoietic response: increased reticulocyte counts (≥300 ppm males, 1,000 ppm females) and hematopoietic cell proliferation in the spleen (≥300 ppm males, 1,000 ppm females). Splenic congestion (1,000 ppm males, 10 ppm and ≥100 ppm females) was also observed and may have been related to the erythron effect.

There were no treatment-related deaths in either sex of mice. The final mean body weight and mean body weight gain of 1,000 ppm male mice were significantly less than those of the control group; the mean body weight gain of 300 ppm female mice was significantly greater than that of the controls. Water consumption by the 1,000 ppm groups was less than that of the control groups during the first week of the study. The liver weights of all exposed groups of male mice and of 300 and 1,000 ppm female mice and the kidney weights of 1,000 ppm males (relative) and females (absolute and relative) were significantly greater than those of the controls. The incidences of hepatocyte centrilobular hypertrophy were significantly increased in the 300 and 1,000 ppm groups of male and female mice, with exposure concentration-related increases in severity. An erythron effect, similar to that observed in rats, was observed in 1,000 ppm female mice only and was characterized by a small decrease in hematocrit value, hemoglobin concentration, and erythrocyte count; no accompanying erythropoietic response was observed in mice.

o-Chloropyridine was mutagenic in *S. typhimurium* strains TA98 and TA100 when tested with exogenous metabolic activation enzymes from rat or hamster liver; no mutagenic activity was observed in the absence of metabolic activation. In vivo, no increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female mice exposed to *o*-chloropyridine for 3 months in drinking water.

Under the conditions of these 3-month drinking water studies, there were treatment-related organ weight changes and lesions in male and female rats and mice. The major target tissues in rats affected by *o*-chloropyridine exposure included the kidney, spleen, bone marrow, and liver; the major target organ in mice was the liver. The measurement most sensitive to *o*-chloropyridine exposure in male rats was increased absolute (all exposure groups) and relative (all exposure groups except 100 ppm) kidney weights in the absence of histopathologic changes [lowest-observed-effect level (LOEL) = 10 ppm]. In female rats, a LOEL of 10 ppm was based on splenic congestion, observed in all treated groups (except 30 ppm), with hematopoietic cell proliferation and pigmentation in the spleen, bone marrow hyperplasia, and hematological changes at higher exposure concentrations. This pattern of erythropoietic responses in the spleen and bone marrow and hematologic changes was also observed in male rats at 300 ppm or greater doses. In male mice, absolute and relative liver weights were significantly higher than controls in all treated groups (LOEL 10 ppm), with histologic changes (centrilobular hepatocyte hypertrophy) occurring at 300 ppm and 1,000 ppm. In female mice, absolute and relative liver weights were significantly higher than controls, with increased incidences of centrilobular hepatocyte hypertrophy occurring at similar exposure concentrations (LOEL 300 ppm).

Synonyms: Alpha-chloropyridine; 2-chloro-(9Cl); 2-chloropyridine (8Cl); pyridines

Summary of Findings Considered to be Toxicologically Relevant in Rats and Mice Exposed to *o*-Chloropyridine in Drinking Water for Three Months

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1/N Mice	Female B6C3F1/N Mice
Concentrations in drinking water	0, 10, 30, 100, 300 or 1,000 ppm	0, 10, 30, 100, 300 or 1,000 ppm	0, 10, 30, 100, 300 or 1,000 ppm	0, 10, 30, 100, 300 or 1,000 ppm
Average daily doses	0, 1, 3, 9, 25, 65 mg/kg	0, 1, 3, 9, 27, 70 mg/kg	0, 1.5, 4.5, 15, 41, 110 mg/kg	0, 1.3, 4, 12, 38, 92 mg/kg
Survival rates	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	10/10, 10/10, 10/10, 10/10, 10/10, 10/10	9/10, 10/10, 10/10, 10/10, 10/10, 10/10
Body weights	↓ 16% compared to controls (1,000 ppm)	↓ 11% compared to controls (1,000 ppm)	↓ 19% compared to controls (1,000 ppm)	↑ 12% compared to controls (300 ppm)
Clinical findings	Thinness (1,000 ppm) in 7/10 on day 8 and 5/10 on day 64	Thinness (1,000 ppm) in 10/10 on day 8	None	None
Organ weights	↑ Absolute and relative kidney weights ↑ Absolute and relative liver weights	↑ Absolute and relative kidney weights ↑ Absolute and relative liver weights	↑ Absolute and relative liver weights	↑ Absolute and relative liver weights ↑ Absolute and relative kidney weights
Clinical pathology (week 14)	↓ Erythrocyte count ↓ Hematocrit ↓ Hemoglobin ↑ Reticulocyte count	↓ Erythrocyte count ↓ Hematocrit ↓ Hemoglobin ↑ Reticulocyte count	None	↓ Erythrocyte count ↓ Hematocrit ↓ Hemoglobin
Reproductive findings	↓ Epididymal sperm count	None	None	None
Nonneoplastic effects	Liver: clear cell focus (0/10, 0/10, 0/10, 0/10, 0/10, 6/10); hepatocyte cytoplasmic vacuolization (0/10, 0/10, 0/10, 0/10, 6/10, 9/10) Spleen: hematopoietic cell proliferation (4/10, 8/10, 6/10, 6/10, 10/10, 10/10); congestion (4/10, 1/10, 2/10, 0/10, 7/10, 10/10) Bone marrow: hyperplasia (1/10, 0/10, 2/10, 1/10, 9/10, 10/10)	Liver: hepatocyte cytoplasmic vacuolization (0/10, 0/10, 0/10, 0/10, 0/10, 9/10) Spleen: hematopoietic cell proliferation (3/10, 3/10, 6/10, 3/10, 7/10, 10/10); congestion (1/10, 7/10, 5/10, 6/10, 6/10, 10/10) Bone marrow: hyperplasia (0/10, 0/10, 0/10, 0/10, 3/10, 9/10)	Liver: centrilobular hepatocyte hypertrophy (0/10, 0/10, 0/10, 1/10, 6/10, 9/10)	Liver: centrilobular hepatocyte hypertrophy (0/10, 0/10, 0/10, 0/10, 4/10, 10/10)
Genetic toxicology				
Bacterial gene mutations:		Positive in *Salmonella typhimurium* strains TA98 and TA100 in the presence of rat or hamster liver S9 activation enzymes; negative in the absence of S9		
Micronucleated erythrocytes				
Mouse peripheral blood in vivo:		Negative in males and females		

Introduction

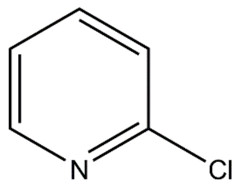

Figure 1. *o*-Chloropyridine (CASRN 109-09-1; Chemical Formula: C_5H_4ClN; Molecular Weight: 113.55)

Synonyms: Alpha-chloropyridine; 2-chloro-(9Cl); 2-chloropyridine (8Cl); pyridines.

Chemical and Physical Properties

o-Chloropyridine is a colorless, oily liquid with a density of 1.205 g/cm^3 at 15°C[1; 2] and a boiling point of 170°C[1]. The water solubility of *o*-chloropyridine is 2.5 g/100 g at 25°C; *o*-chloropyridine is soluble in alcohol and ether[2; 3]. *o*-Chloropyridine poses a slight fire hazard, and when exposed to heat can release toxic phosgene gas[1; 4].

Production, Use, and Human Exposure

o-Chloropyridine can be prepared by direct chlorination of pyridine in the vapor phase (>300°C) in the presence of a diluent[5]. One patented process (Reilly Industries, Inc.) involves selective chlorination of pyridine with chlorine in nitrogen in the presence of water vapor; synthesis occurs in two stages at 470° and 290°C[6]. *o*-Chloropyridine is prepared for use as an intermediate by heating potassium pyrrole with chloroform. Another patented process involves the reaction of alpha-picoline with chlorine in the gas phase in the presence of water and a catalyst, such as pyrophyllite; this process yields a mix of chlorinated pyridines[7]. A third patented process reacts 2-hydroxypyridine with phosgene in the presence of an amide, such as N,N-dimethylformamide[8].

o-Chloropyridine is listed as a chemical in commerce in the United States; no production or sales quantities were listed to avoid disclosure of the single producer's operations[9]. Companies are required to report production of 10,000 pounds or more or sales of $10,000 or more. The United States Environmental Protection Agency[10] lists *o*-chloropyridine in its Toxic Substances Control Act inventory. In 2006, the production and import of *o*-chloropyridine in the United States was reported to range from 1 million to 10 million pounds[11].

o-Chloropyridine is used as an intermediate in synthetic organic, pharmaceutical, and agricultural chemical (fungicides, herbicides) manufacturing. It is also used as a catalyst for phase transfer[1; 12]. It is a key intermediate in the manufacture of pyrithione-based biocides for use in cosmetics and various pharmaceutical products[13]. *o*-Chloropyridine is used as a starting material in the production of the antihistamine, pheniramine, and the antiarrhythmic, diisopyramide[5]. *o*-Chloropyridine is available in purified (99%), technical (95%), or crude (80%) grades[3; 12; 14-16].

There is potential for occupational exposure to *o*-chloropyridine during its production and use as an industrial chemical intermediate. A survey of the Olin Corporation plant in Rochester, NY, noted that significant exposures to chemicals in the *o*-chloropyridine processing area existed and

that high vapor concentrations resulting from liquid spills and minor leaks were detected in the closed processing area, and quarterly sampling of personnel to detect exposures was recommended[17]. *o*-Chloropyridine is not listed in the National Occupational Exposure Survey[18].

Gehring et al.[19] noted that the solubility of *o*-chloropyridine in organic solvents suggests that it might have high dermal absorption and subsequently, Gehring[4] reported experimental evidence that chlorinated pyridines are rapidly absorbed through intact skin.

o-Chloropyridine has not been reported to occur naturally; however, it is reported to be an environmental contaminant. The Dow Chemical Company has identified it as a trace organic chemical in process streams and wastewater[20]. It has also been identified as a Rhine River pollutant in the Netherlands (0.023 µg/L) and a trace organic contaminant (detected, not quantified) in drinking water derived from river water in Barcelona, Spain[21; 22].

o-Chloropyridine was detected as an intermediate product in amended freshwater sediment slurries; it arose from the biotransformation of 2,3-dichloropyridine under anaerobic (methanogenic) conditions. It was reported to be persistent and not to be further metabolized during a 6-month incubation period[23]. Adrian and Suflita[24] reported that *o*-chloropyridine resisted biodegradation in anoxic aquifer slurries incubated for 11 months.

Regulatory Status

No standards or guidelines have been set by the National Institute for Occupational Safety and Health or the Occupational Safety and Health Administration for occupational exposure or workplace maximum allowable levels of *o*-chloropyridine. The American Conference of Governmental Industrial Hygienists has not recommended a threshold limit value or biological exposure index for this compound[25]. *o*-Chloropyridine is classified as a poisonous material by the U.S. Department of Transportation[26]. The USEPA[17] has issued a requirement for health and safety data reporting on pyridine and pyridine derivatives, including *o*-chloropyridine.

Absorption, Distribution, and Metabolism

Incubation of *o*-chloropyridine with liver homogenate and cofactors yielded *o*-chloropyridine-N-oxide and pyridine-N-oxide[27]. No disposition or metabolism studies of *o*-chloropyridine in experimental animals or humans could be found in the literature.

Toxicity

Experimental Animals

The acute toxicity of *o*-chloropyridine has been studied in mice, rats, and rabbits[19]. The mouse oral LD50 is 100 mg/kg, and the intraperitoneal LD50 is 130 mg/kg. Gross lesions included swollen and fatty livers as well as hemorrhage and necrosis at higher doses and swollen, edematous kidneys in some animals. Concurrent administration of methionine had a protective effect against toxicity, while cysteine and nicotinamide enhanced the toxicity of *o*-chloropyridine. In rabbits, *o*-chloropyridine was essentially as toxic when applied to the skin as when given by intraperitoneal injection. The rabbit dermal LD50 is 64 mg/kg and the intraperitoneal LD50 is 48 mg/kg. The primary gross lesion, regardless of route of administration, was hemorrhagic necrosis of the liver. Instillation of undiluted or

10% *o*-chloropyridine solution in propylene glycol in the eyes of rabbits caused severe inflammation of the conjunctiva and moderate clouding of the cornea that persisted for 48 hours.

Humans

o-Chloropyridine was reported to be a skin irritant and toxic by ingestion[1; 14].

Reproductive and Developmental Toxicity

No reproductive or developmental toxicity studies of *o*-chloropyridine in animals or humans were identified in the literature.

Carcinogenicity

No 2-year carcinogenicity studies of *o*-chloropyridine in animals or epidemiological studies or case reports investigating the association of exposure to *o*-chloropyridine and cancer risk in humans were identified in the literature.

Genetic Toxicity

o-Chloropyridine was mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, and TA102 in tests conducted with exogenous metabolic activation (S9 liver enzymes), but no mutagenicity was detected in any of the strains without activation[27; 28]. Zimmerman et al.[29] reported weak induction of aneuploidy in the yeast Saccharomyces cerevisiae treated with *o*-chloropyridine concentrations up to 0.50%.

Tests in mammalian cells showed no induction of chromosomal aberrations by *o*-chloropyridine in African green monkey kidney cells (V3) in the absence of S9 enzymes[30]. However, when V3 cells were treated with 1,600 µg/mL *o*-chloropyridine in the presence of pyridine N-oxide, 25% of the cells were chromosomally aberrant; pyridine N-oxide (200 µg/mL) alone was not clastogenic in V3 cells[30].

In contrast to the results in V3 cells, *o*-chloropyridine was reported to induce gene mutations, chromosomal aberrations, and micronuclei in L5178Y mouse lymphoma cells with and without metabolic activation (rat liver S9 enzymes); the gene mutation and chromosomal aberration responses were stronger in the presence of S9[31]. These results led Dearfield et al.[31] to postulate that substitution at the *o*-position promotes genotoxic effects of the halogenated pyridines through N-oxidation by microsomal enzymes. This mechanism was also explored in experiments conducted by Chłopkiewicz et al.[27] and designed to investigate the effects of N-oxidation and OH radicals on the mutagenicity of *o*-chloropyridine in *S. typhimurium* TA100; they reported mutagenicity in a plate incorporation method in TA100 only with S9 and no, or greatly reduced, mutagenicity when glutathione, thiourea, D-mannitol, or pyridine N-oxide was added to the test mixture.

The metabolite *o*-chloropyridine N-oxide was not mutagenic in *S. typhimurium* strain TA100, with or without S9, when tested using a plate incorporation protocol[27].

The structurally related compound, 3-chloropyridine, was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, or TA102, with or without S9 metabolic activation[28; 32]. 3-Chloropyridine induced a dose-related increase in chromosomal aberrations in V3 cells in the

absence of S9[30]; clastogenicity was eliminated when cultures were incubated in the presence of pyridine N-oxide. 3-Chloropyridine induced small increases in gene mutations and chromosomal aberrations in mouse lymphoma L5178Y cells[31], with and without S9 metabolic activation; micronuclei were induced by 3-chloropyridine only in the absence of S9.

Study Rationale

o-Chloropyridine was nominated for testing by NTP based on increasing production and use as a site-limited pharmaceutical and agrochemical intermediate, the potential for occupational and environmental exposures during its manufacture, its persistence in the environment (lasting longer than 6 months), evidence of mutagenicity based on results of several short-term test systems, and suspicion of carcinogenicity based on structure and evidence of mutagenic or carcinogenic effects associated with structurally related chemicals. Dermal exposure was selected for the 2-week studies based on the likely route of occupational exposure. The route of exposure was changed to oral, via drinking water, in the 3-month studies due to concerns regarding skin irritation following longer dermal exposure and to allow comparison of *o*-chloropyridine toxicity with the results of the NTP 3-month drinking water studies of a structurally similar compound, pyridine[33].

Materials and Methods

Procurement and Characterization

o-Chloropyridine

o-Chloropyridine was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (15306CN) that was used in the 2-week dermal and 3-month drinking water studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH, and Richland, WA), Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO), Galbraith Laboratories, Inc. (Knoxville, TN), and the study laboratory, BioReliance Corporation (Rockville, MD) (Appendix F). Reports on analyses performed in support of the *o*-chloropyridine studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear colorless liquid, was identified as *o*-chloropyridine by infrared (IR) and proton and carbon-13 nuclear magnetic resonance spectroscopy. The purity of lot 15306CN was determined by elemental analyses and two gas chromatography (GC) systems. Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for *o*-chloropyridine. GC by both systems indicated one major peak and two impurities with areas at least 0.1% relative to the major peak area. The overall purity of lot 15306CN was determined to be 99% or greater.

To ensure stability, the bulk chemical was stored at room temperature in sealed amber glass bottles under a headspace of inert gas, protected from light and moisture. Periodic reanalyses of the bulk chemical were performed during the 2-week and 3-month studies using GC, and no degradation of the bulk chemical was detected.

Ethanol

95% Ethanol was obtained from Clear Spring Distilling Company (Clearwater, KY) in one lot (21049312) that was used as the dosing vehicle in the 2-week dermal studies. Lot 21049312, a clear liquid, was identified as ethanol by IR spectroscopy. The purity of lot 21049312 was determined using GC. Analysis indicated one major peak and three impurities each with a relative concentration of less than or equal to 0.0001%.

Preparation and Analysis of Dose Formulations

Dermal Studies

The dose formulations were prepared by mixing *o*-chloropyridine with 95% ethanol (Table F-2). Stability studies of a 3.125 mg/mL dose formulation were performed by the analytical chemistry laboratory with GC. Stability was confirmed for at least 43 days for dose formulations stored in sealed amber glass vials at room temperature, 5°C, or −20°C. Data from a simulated animal room stability study indicated that *o*-chloropyridine is stable dissolved in 95% ethanol when exposed to light for up to 3 hours at room temperature. The dose formulations were stored under a headspace of inert gas in refrigerated amber glass vials with Teflon®-lined lids for up to 23 days.

The dose formulations were analyzed on the day they were prepared by the study laboratory using GC. All seven dose formulations analyzed for rats and mice were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; one of five formulations for rats and all five formulations for mice were within 10% of the target concentrations. High concentrations measured for some of the rat animal room samples were attributed to improper sealing of the vials after dosing and possible solvent evaporation.

Drinking Water Studies

The dose formulations were prepared by mixing *o*-chloropyridine with tap water (Table F-2). Stability studies of the 10 ppm dose formulation were performed by the analytical chemistry laboratory with high-performance liquid chromatography (HPLC). Stability was confirmed for at least 43 days for dose formulations stored in sealed polyethylene bottles protected from light at 5°C and for at least 8 days under simulated animal room conditions. The dose formulations were stored refrigerated in Cubitainers® with taps, protected from light, for up to 21 days.

The dose formulations were analyzed at the beginning, midpoint, and end of the studies by the study laboratory using HPLC; animal room samples of these dose formulations were also analyzed. All 15 dose formulations analyzed and used for rats and mice were within 10% of the target concentrations. Of the animal room samples analyzed, all 15 for rats and 12 of 15 for mice were within 10% of the target concentrations, and the remaining three were less than 12% of the target concentration.

Animal Welfare

Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. 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 BioReliance Corporation Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines.

Two-week Dermal Studies

Male and female F344/N rats and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 12 (rats) or 13 (mice) days and were 5 to 6 weeks old on the first day of the studies. *o*-Chloropyridine in 95% ethanol was applied using automatic (adjustable volume) pipettes to the center of a shaved area of skin on the dorsal surface from just posterior to the scapulae to the base of the tail; the shaved area was larger than the application site. A constant concentration of test chemical per dose concentration was administered to each animal at volumes of 0.5 mL/kg body weight for rats and 2 mL/kg for mice. Five male and five female rats and mice per dose group were administered *o*-chloropyridine in ethanol 5 days per week over a 16-day period (12 dose days). Rats and mice were administered 0, 6.25, 12.5, 25, 50, or 100 mg *o*-chloropyridine/kg body weight. Vehicle control animals were administered ethanol alone. Feed and water were available ad libitum. Rats and mice were housed individually. Clinical findings were recorded daily. The animals were weighed initially, on day 8, and at the end of the studies. Prior to dosing and at the start of the studies, five male and five

female rats and mice were selected for parasite evaluation and gross observation for disease presence. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on gross lesions in rats; no gross lesions were observed in mice.

Three-month Drinking Water Studies

For the 3-month studies, the route of exposure was changed from dermal administration to drinking water, allowing for comparison of *o*-chloropyridine toxicity with the results of the NTP 3-month drinking water studies of a structurally similar compound, pyridine[33]. Male and female F344/N rats and B6C3F1/N mice were obtained from the NTP colony maintained at Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 3 weeks old. Animals were quarantined for 11 to 12 days (rats) or 14 to 15 days (mice); rats were 5 to 6 weeks old and mice were 6 to 7 weeks old on the first day of the studies. Upon arrival at the facility, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Additionally, the health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I). All test results were negative.

The core study animals consisted of groups of 10 male and 10 female rats and mice that were exposed to *o*-chloropyridine in drinking water at concentrations of 0, 10, 30, 100, 300, or 1,000 ppm for 14 weeks. Exposure concentrations for the 3-month drinking water studies were selected for comparison to the 3-month pyridine study results[33]. Additional groups of 10 male and 10 female rats designated as clinical pathology study rats were exposed to the same concentrations for 22 days for evaluation of clinical pathology endpoints at days 4 and 22; these animals were not assessed for other endpoints. Clinical pathology results presented for week 14 are from core study animals. Feed and water were available ad libitum. Rats and female mice were housed five per cage and male mice were housed individually. Core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Water consumption by core study animals was recorded weekly by cage. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with a 70% carbon dioxide:30% oxygen mixture, and blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 22 and from core study rats and mice at the end of the 3-month studies for hematology and clinical chemistry (rats only) analyses (Appendix B). Hematology and clinical chemistry parameters were measured using a ABX Pentra C+ Analyzer (Horiba Instruments Corporation, Irvine, CA) and a Hitachi 717 Analyzer (Boehringer Mannheim, Indianapolis, IN), respectively, using reagents supplied by the manufacturers. The parameters measured are listed in Table 1.

At the end of the 3-month drinking water studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice exposed to 0, 100, 300, or 1,000 ppm. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were 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 rats and mice. 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 (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. Complete histopathologic examinations were performed by the study laboratory pathologist on core study rats and mice in the 0 and 1,000 ppm groups; the bone marrow, kidney, liver, and spleen of rats and the kidney and liver of mice were examined in the remaining core study groups. If findings were observed in the 1,000 ppm group, a read down was performed in which lower exposure groups were examined until a no-effect level was determined for a given endpoint. Table 1 lists the tissues and organs routinely examined.

After a review of the laboratory reports and selected histopathology slides by a quality assessment (QA) 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 QA pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus of the PWG or a consensus between the study laboratory pathologist, NTP pathologist, QA pathologist(s), and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman[34] and Boorman et al.[35].

Table 1. Experimental Design and Materials and Methods in the Studies of *o*-Chloropyridine

Two-week Dermal Studies	Three-month Drinking Water Studies
Study Laboratory	
BioReliance Corp. (Rockville, MD)	BioReliance Corp. (Rockville, MD)
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)

Two-week Dermal Studies	Three-month Drinking Water Studies
Time Held Before Studies	
Rats: 12 days Mice: 13 days	Rats: 11 (males) or 12 (females) days Mice: 14 (males) or 15 (females) days
Average Age When Studies Began	
5–6 weeks	Rats: 5–6 weeks Mice: 6–7 weeks
Date of First Dose or Exposure	
Rats: September 19, 2000 Mice: September 20, 2000	Rats: July 28, 2003 Mice: July 31, 2003
Duration of Dosing or Exposure	
5 exposures per week for 16 days	14 weeks (core study)
Date of Last Dose or Exposure	
Rats: October 4, 2000 Mice: October 5, 2000	Rats: October 27 (males) or 28 (females), 2003 Mice: October 30 (males) or 31 (females), 2003
Necropsy Dates	
Rats: October 5, 2000 Mice: October 6, 2000	Rats: October 27 (males) or 28 (females), 2003 Mice: October 30 (males) or 31 (females), 2003
Average Age at Necropsy	
8–9 weeks	Rats: 18–19 weeks Mice: 19–20 weeks
Size of Study Groups	
5 males and 5 females	10 males and 10 females
Method of Distribution	
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies
Animals per Cage	
1 animal	Rats: 5 Mice: 1 (males) or 5 (females)
Method of Animal Identification	
Tail tattoo	Tail tattoo
Diet	
NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available ad libitum, changed once weekly	Same as 2-week studies
Water	
Tap water (Washington, D.C., Suburban Sanitary Commission Potomac Plant) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available ad libitum	Same as 2-week studies, except via amber glass bottles equipped with stainless steel sipper tubes and neoprene stoppers with screw on polypropylene lids

Two-week Dermal Studies	Three-month Drinking Water Studies
Cages	
Polycarbonate (Lab Products, Inc., Seaford, DE), changed once weekly	Same as 2-week studies; changed twice weekly for rats and female mice and once weekly for male mice; rotated every 2 weeks
Bedding	
Irradiated, heat-treated Sani-Chips® (P.J. Murphy Forest Products Corp., Montville, NJ), changed once weekly	Same as 2-week studies; changed twice weekly for rats and female mice and once weekly for male mice
Cage Filters	
Remay® 2016 (Snow Filtration, Cincinnati, OH)	Same as 2-week studies
Racks	
Stainless steel (Lab Products, Inc., Seaford, DE), changed once every 2 weeks	Same as 2-week studies; changed and rotated once every 2 weeks
Animal Room Environment	
Temperature: 72° ± 3°F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Same as 2-week studies
Doses or Exposure	
0, 6.25, 12.5, 25, 50, or 100 mg/kg	0, 10, 30, 100, 300, or 1,000 ppm
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; core study animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Water consumption by core study animals was recorded weekly by cage.
Method of Sacrifice	
Carbon dioxide asphyxiation	Same as 2-week studies
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.
Clinical Pathology	
None	Blood was collected from the retroorbital sinus of clinical pathology study animals on days 4 and 22 and from core study animals at the end of the studies for hematology and clinical chemistry (rats only). ***Hematology***: hematocrit; hemoglobin; erythrocyte, reticulocyte, nucleated erythrocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. ***Clinical chemistry***: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids

Two-week Dermal Studies	Three-month Drinking Water Studies
Histopathology	
Gross lesions were examined.	Complete histopathology was performed on 0 and 1,000 ppm core study rats and mice and a read down was performed when needed. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with mainstem bronchus), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis (with epididymis), thymus, thyroid gland, trachea, urinary bladder, and uterus. The bone marrow, kidney, liver, and spleen were examined in the remaining groups of core study rats, and the kidney and liver were examined in the remaining groups of mice.
Sperm Motility and Vaginal Cytology	
None	At the end of the studies, sperm samples were collected from core study males in the 0, 100, 300, and 1,000 ppm groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. 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 core study females exposed to 0, 100, 300, or 1,000 ppm for vaginal cytology evaluations.

Statistical Methods

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendix A as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test[36], a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed or exposed 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[37] and Williams[38; 39]. Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley[40] (as modified by Williams[41]), and Dunn[42]. Jonckheere's test[43] 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[44] were examined by NTP personnel, and implausible values were eliminated from the analysis. Proportions of regular cycling females in each exposed group were compared to the control group using the Fisher exact test[36]. 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[45]. For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within each stage as well as for skipping estrus or diestrus within a cycle. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Quality Assurance Methods

The 2-week and 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations[46]. In addition, as records from the 3-month studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent QA contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Toxicity Study 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 Toxicity Study Report.

Genetic Toxicology

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed as reported by Zeiger et al.[47]. *o*-Chloropyridine was sent to the laboratory as a coded aliquot. It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 either in buffer or 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.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of *o*-chloropyridine. The assay limit dose was 10,000 µg/plate. All positive trials were repeated under the conditions that elicited the positive response.

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.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor et al.[48]. At the end of the 3-month toxicity 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 stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic (mature) erythrocytes (NCEs) in each of five animals per exposure group. In addition, the percentage of polychromatic erythrocytes (PCEs; reticulocytes) in a population of 1,000 erythrocytes was scored for each dose 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 with 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. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

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 samples of a chemical were tested in the same assay, and different results were obtained among these samples 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 Toxicity Study Report presents a result that represents a scientific judgment of the overall evidence for activity of the chemical in an assay.

Results

Rats

Two-week Dermal Study

All rats survived to the end of the study (Table 2). The final mean body weights and body weight gains of all dosed groups were similar to those of the vehicle control groups. There were no clinical findings related to *o*-chloropyridine administration.

The absolute and relative liver weights of 50 (absolute, 10.6%) and 100 mg/kg (absolute, 9.6%) males were significantly greater than those of the vehicle controls (Table 3 and Table C-1). Absolute and relative organ weights of all dosed groups of females were similar to those of the vehicle controls (Table C-1). No gross or microscopic lesions were considered related to *o*-chloropyridine administration.

Table 2. Survival and Body Weights of Rats in the Two-week Dermal Study of *o*-Chloropyridine[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	90 ± 3	157 ± 6	68 ± 3	
6.25	5/5	88 ± 4	159 ± 6	71 ± 3	101
12.5	5/5	87 ± 3	157 ± 4	70 ± 2	100
25	5/5	85 ± 3	153 ± 3	68 ± 2	97
50	5/5	88 ± 2	158 ± 5	71 ± 3	101
100	5/5	87 ± 3	158 ± 4	71 ± 2	101
Female					
0	5/5	82 ± 3	120 ± 2	39 ± 2	
6.25	5/5	80 ± 2	121 ± 1	41 ± 2	101
12.5	5/5	81 ± 3	120 ± 3	40 ± 1	100
25	5/5	81 ± 4	122 ± 4	41 ± 2	102
50	5/5	80 ± 2	120 ± 2	40 ± 2	100
100	5/5	80 ± 2	120 ± 2	40 ± 1	100

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at 17 days/number initially in group.

Table 3. Liver Weights and Liver-Weight-to-Body-Weight Ratios for Male Rats in the Two-week Dermal Study of *o*-Chloropyridine[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Necropsy body wt	157 ± 6	159 ± 6	157 ± 4	153 ± 3	158 ± 5	158 ± 4
Liver						
Absolute	8.02 ± 0.28	8.13 ± 0.18	8.25 ± 0.23	8.03 ± 0.16	8.87 ± 0.35*	8.79 ± 0.11*
Relative	51.083 ± 0.188	51.251 ± 0.857	52.473 ± 1.018	52.428 ± 0.422	55.961 ± 0.567**	55.732 ± 0.777**

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

Exposure concentration selection rationale: Exposure concentrations in the 3-month drinking water study were selected for comparison to the 3-month pyridine study results[33].

Three-month Drinking Water Study

All rats survived to the end of the study (Table 4). Final mean body weights and body weight gains of male and female rats exposed to 1,000 ppm were significantly less than those of the controls (Table 4 and Figure 2). Water consumption was lower in the 1,000 ppm groups, compared to controls, particularly during week 1 of the study. In male and female rats, water consumption in the 1,000 ppm groups was approximately half that of controls in the first week, increasing in week 2. By week 14, water consumption by female rats had completely recovered while water consumption by male rats was only slightly less than that of controls (Table 4, Table G-1, and Table G-2). Drinking water concentrations of 10, 30, 100, 300, and 1,000 ppm resulted in average daily doses of approximately 1, 3, 9, 25, and 65 mg *o*-chloropyridine/kg body weight to males and 1, 3, 9, 27, and 70 mg/kg to females. In the 1,000 ppm groups, thinness was noted in seven of 10 males and all females on day 8 and also in five of 10 males on day 64; thinness noted on day 8 was likely due to dehydration from reduction in water consumption. Thinness was diagnosed based on the degree of visibility of the vertebrae and pelvic bones.

Table 4. Survival, Body Weights, and Water Consumption of Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

Concentration (ppm)	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 14
Male							
0	10/10	90 ± 2	320 ± 6	230 ± 5		14.7	17.3
10	10/10	93 ± 2	326 ± 7	233 ± 6	100	15.5	17.7
30	10/10	92 ± 2	326 ± 8	234 ± 7	102	15.1	13.6
100	10/10	91 ± 2	326 ± 8	235 ± 9	102	15.4	18.1
300	10/10	91 ± 2	309 ± 7	218 ± 7	97	13.6	17.5
1,000	10/10	94 ± 3	268 ± 6**	173 ± 4**	84	6.5	15.1
Female							
0	10/10	86 ± 2	195 ± 4	109 ± 3		12.5	13.4
10	10/10	88 ± 2	200 ± 2	112 ± 3	103	13.8	17.7
30	10/10	87 ± 1	200 ± 4	113 ± 4	103	13.0	14.8
100	10/10	88 ± 2	196 ± 2	108 ± 3	101	12.6	17.4
300	10/10	86 ± 2	190 ± 3	104 ± 3	97	12.2	12.7
1,000	10/10	87 ± 2	174 ± 2**	87 ± 3**	89	6.1	15.8

**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.

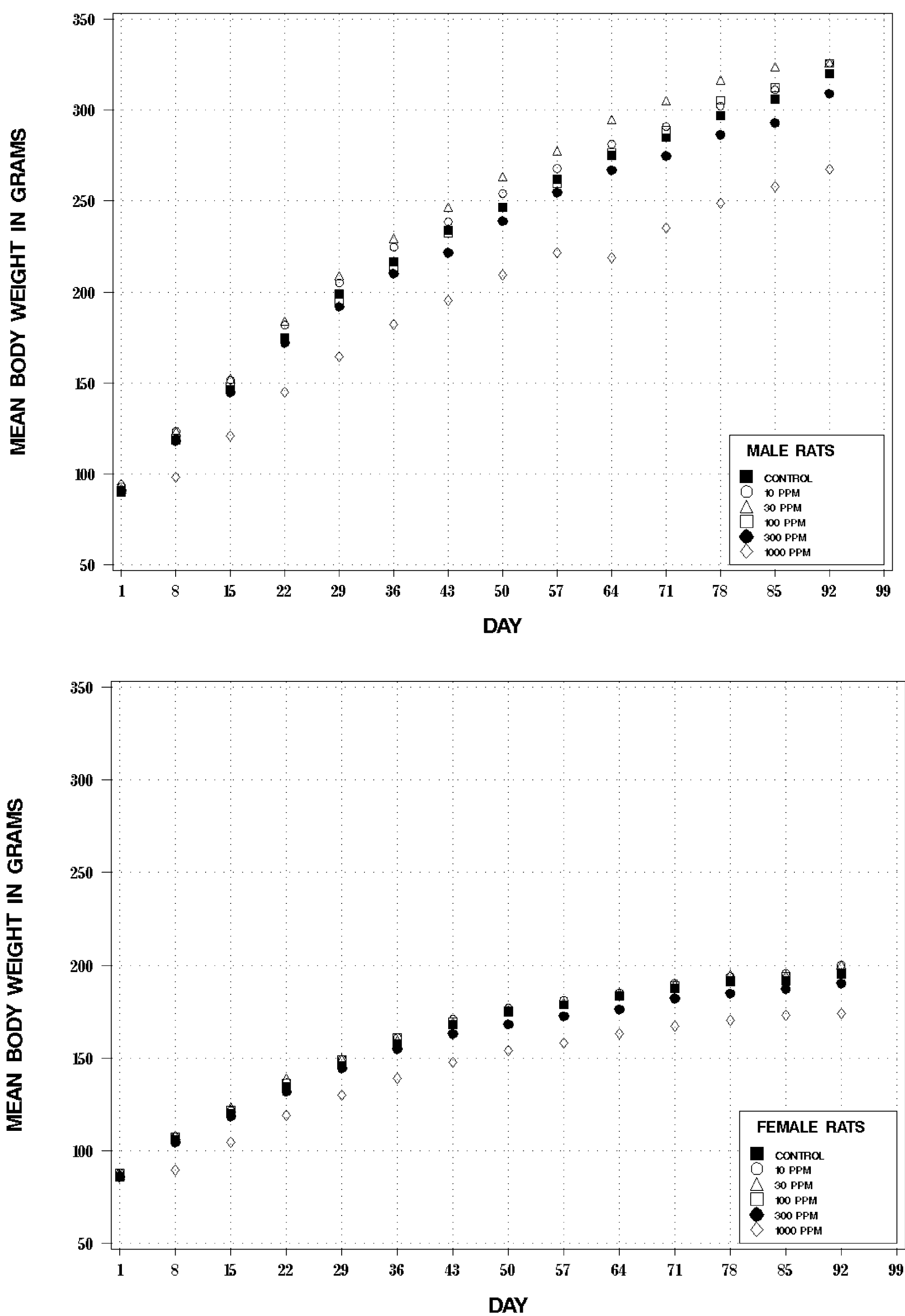

Figure 2. Growth Curves for Rats Exposed to *o*-Chloropyridine in Drinking Water for Three Months

On day 4, the hematology findings demonstrated a small (approximately 10%) increase in the circulating erythron (i.e., an erythrocytosis). The erythrocytosis was evidenced by increases in

hematocrit values, hemoglobin concentrations, and erythrocyte counts of the 1,000 ppm males (Table 5 and Table B-1). Additionally, urea nitrogen concentrations increased in the 1,000 ppm males on day 4 (Table B-1). These early changes may be the result of dehydration; there was an approximate 50% reduction in water intake during week 1 of the study (Table 4). The increases in the erythron and serum urea nitrogen were transient and disappeared by day 22, being replaced by an apparent dose-related erythron decrease in male and female rats exposed to 100 ppm or greater. The erythron decrease was small (≤10%) and was demonstrated by decreased hematocrit values and hemoglobin concentrations. The erythrocyte counts were unaffected in this case; however, there were small reductions (≤5%) in erythrocyte size (microcytosis), evidenced by decreases in the mean cell volume. By week 14, the erythron decrease had progressed to include decreases in the erythrocyte count and increases in reticulocyte count in the 300 ppm male and 1,000 ppm male and female rats. While the magnitude of these changes were small, they were supported by histologic changes in the bone marrow and spleen.

An exposure concentration-related hyperalbuminemia, demonstrated by increased serum albumin concentrations, occurred in the 30 ppm or greater males and females at week 14; the 1,000 ppm groups demonstrated a 10% to 15% increase (Table 5 and Table B-1). The hyperalbuminemia was progressive and, on day 4, involved a minimal 5% increase in the 1,000 ppm males only. By day 22, the 100, 300, and 1,000 ppm males and females were affected. At week 14, the increased serum albumin was accompanied by proportional increases in the serum total protein concentration. There was also evidence of a potential alteration in hepatic function demonstrated by increased serum bile acid concentrations. The most consistent change occurred on day 4, involving the 300 and 1,000 ppm males and females. Though the mechanism was unknown, the effect leading to the bile acid increase was transient in males, and bile acid concentrations demonstrated amelioration by day 22 and were essentially resolved at week 14. Bile acid concentrations remained higher in female mice at week 14 compared to controls.

Table 5. Selected Clinical Pathology Data for Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
Hematology						
n						
Day 4	10	10	10	9	9	10
Day 22	10	10	10	10	10	10
Week 14	9	10	10	10	10	10
Hematocrit (%)						
Day 4	40.0 ± 0.4	39.8 ± 0.4	40.5 ± 0.6	38.8 ± 0.5	39.6 ± 0.5	43.3 ± 0.9*
Day 22	43.4 ± 0.4	42.7 ± 0.4	42.6 ± 0.4	41.2 ± 0.6**	41.5 ± 0.5**	39.6 ± 0.4**
Week 14	45.5 ± 0.6	45.2 ± 0.3	46.3 ± 0.3	44.5 ± 0.9	42.9 ± 0.4**	41.7 ± 0.4**
Hemoglobin (g/dL)						
Day 4	13.4 ± 0.1	13.4 ± 0.1	13.6 ± 0.2	13.1 ± 0.2	13.3 ± 0.2	14.5 ± 0.3*
Day 22	14.8 ± 0.1	14.5 ± 0.1	14.5 ± 0.1	14.1 ± 0.2**	14.1 ± 0.2**	13.5 ± 0.1**
Week 14	15.2 ± 0.2	15.1 ± 0.1	15.4 ± 0.1	15.0 ± 0.3	14.4 ± 0.1**	13.9 ± 0.1**
Erythrocytes (10^6/µL)						
Day 4	6.80 ± 0.08	6.85 ± 0.10	6.92 ± 0.10	6.72 ± 0.09	6.95 ± 0.10	7.53 ± 0.14**
Day 22	7.50 ± 0.07	7.35 ± 0.09	7.37 ± 0.08	7.18 ± 0.11*	7.30 ± 0.08	7.25 ± 0.08
Week 14	8.91 ± 0.11	8.75 ± 0.07	9.04 ± 0.08	8.76 ± 0.15	8.51 ± 0.06**	8.42 ± 0.09**
Reticulocytes (10^6/µL)						
Day 4	0.46 ± 0.04	0.45 ± 0.04	0.54 ± 0.02	0.50 ± 0.05	0.46 ± 0.03	0.39 ± 0.02
Day 22	0.33 ± 0.04	0.33 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.36 ± 0.04	0.41 ± 0.02
Week 14	0.26 ± 0.01	0.27 ± 0.02	0.27 ± 0.02	0.25 ± 0.02	0.37 ± 0.02**	0.32 ± 0.03*
Mean cell volume (fL)						
Day 4	58.8 ± 0.3	58.1 ± 0.2	58.5 ± 0.3	57.8 ± 0.3*	56.9 ± 0.2**	57.5 ± 0.2**
Day 22	57.9 ± 0.3	58.0 ± 0.3	57.7 ± 0.2	57.3 ± 0.3	56.8 ± 0.3**	54.7 ± 0.2**
Week 14	51.2 ± 0.2	51.6 ± 0.2	51.3 ± 0.2	50.8 ± 0.3	50.5 ± 0.2*	49.3 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.1	19.5 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.1 ± 0.1**	19.3 ± 0.1**
Day 22	19.8 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.4 ± 0.0**	18.7 ± 0.1**
Week 14	17.0 ± 0.0	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	16.9 ± 0.1	16.5 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.6 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.7 ± 0.1	33.6 ± 0.1	33.5 ± 0.1
Day 22	34.1 ± 0.1	34.1 ± 0.1	34.0 ± 0.1	34.2 ± 0.1	34.1 ± 0.1	34.1 ± 0.1
Week 14	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.4 ± 0.1

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Clinical Chemistry						
n	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
Day 22	6.4 ± 0.0	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.8 ± 0.1*	6.6 ± 0.1
Week 14	7.3 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.8 ± 0.1**	8.0 ± 0.1**	8.3 ± 0.1**
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.3 ± 0.1*
Day 22	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.0**	4.7 ± 0.1**	4.7 ± 0.1**
Week 14	4.9 ± 0.0	4.9 ± 0.0	5.0 ± 0.0*	5.2 ± 0.1**	5.4 ± 0.1**	5.7 ± 0.0**
Bile acids (µmol/L)						
Day 4	3.8 ± 1.4	26.5 ± 2.1	26.4 ± 1.3	33.8 ± 2.2**	49.8 ± 6.4**	41.0 ± 5.4**
Day 22	25.7 ± 1.1	25.5 ± 1.2	31.0 ± 2.6	28.0 ± 2.0	34.9 ± 2.9**	39.1 ± 2.8**
Week 14	31.1 ± 3.4	26.0 ± 1.0	29.0 ± 1.9	26.1 ± 0.8	33.1 ± 3.1	39.2 ± 2.9
Female						
n						
Day 4	10	9	10	10	10	9
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	42.6 ± 0.5	41.4 ± 0.5	42.6 ± 0.4	40.6 ± 1.1	39.8 ± 0.8*	44.9 ± 0.8
Day 22	44.9 ± 0.3	45.9 ± 0.4	44.4 ± 0.4	43.5 ± 0.3*	42.4 ± 0.6**	42.4 ± 1.1**
Week 14	44.3 ± 0.8	45.2 ± 0.3	45.1 ± 0.3	43.7 ± 0.7	42.6 ± 0.2**	40.7 ± 0.4**
Hemoglobin (g/dL)						
Day 4	14.4 ± 0.2	14.1 ± 0.2	14.4 ± 0.2	13.8 ± 0.4	13.6 ± 0.3	15.3 ± 0.3
Day 22	15.5 ± 0.1	15.9 ± 0.1	15.4 ± 0.1	15.2 ± 0.1	14.8 ± 0.2*	14.7 ± 0.4**
Week 14	15.1 ± 0.2	15.4 ± 0.1	15.4 ± 0.1	15.0 ± 0.2	14.7 ± 0.1*	13.9 ± 0.2**
Erythrocytes (10^6/µL)						
Day 4	7.33 ± 0.09	7.16 ± 0.09	7.35 ± 0.07	7.02 ± 0.19	6.93 ± 0.15	7.87 ± 0.13
Day 22	7.80 ± 0.07	7.95 ± 0.08	7.75 ± 0.08	7.70 ± 0.06	7.51 ± 0.13	7.74 ± 0.17
Week 14	8.30 ± 0.13	8.50 ± 0.05	8.48 ± 0.07	8.27 ± 0.12	8.17 ± 0.03	7.90 ± 0.07**
Reticulocytes (10^6/µL)						
Day 4	0.36 ± 0.01	0.35 ± 0.03	0.35 ± 0.01	0.36 ± 0.02	0.32 ± 0.02	0.28 ± 0.01**
Day 22	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.22 ± 0.03	0.29 ± 0.02*
Week 14	0.23 ± 0.02	0.26 ± 0.02	0.21 ± 0.02	0.23 ± 0.02	0.26 ± 0.03	0.33 ± 0.02**

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Mean cell volume (fL)						
Day 4	58.0 ± 0.2	57.8 ± 0.2	57.8 ± 0.3	57.8 ± 0.3	57.6 ± 0.2	57.1 ± 0.2**
Day 22	57.5 ± 0.2	57.7 ± 0.2	57.4 ± 0.3	56.6 ± 0.2*	56.6 ± 0.4*	54.6 ± 0.2**
Week 14	53.5 ± 0.2	53.2 ± 0.1	53.2 ± 0.1	52.8 ± 0.1**	52.1 ± 0.2**	51.5 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.4 ± 0.1
Day 22	19.9 ± 0.1	20.0 ± 0.1	19.9 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	19.0 ± 0.1**
Week 14	18.2 ± 0.1	18.1 ± 0.1	18.1 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	17.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.8 ± 0.1	34.0 ± 0.1	33.8 ± 0.1	34.1 ± 0.1	34.3 ± 0.1*	34.0 ± 0.1
Day 22	34.5 ± 0.1	34.6 ± 0.1	34.7 ± 0.1	35.0 ± 0.1*	34.8 ± 0.1	34.7 ± 0.1
Week 14	34.1 ± 0.2	34.1 ± 0.1	34.1 ± 0.1	34.4 ± 0.1	34.5 ± 0.2	34.0 ± 0.1
Clinical Chemistry						
n	10	10	10	10	10	10
Total protein (g/dL)						
Day 4	5.9 ± 0.1	5.8 ± 0.0	6.0 ± 0.1	5.9 ± 0.0	5.9 ± 0.1	5.8 ± 0.1
Day 22	6.3 ± 0.1	6.6 ± 0.1	6.5 ± 0.2	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
Week 14	6.9 ± 0.1	7.2 ± 0.1	7.5 ± 0.1**	7.4 ± 0.1**	7.5 ± 0.1**	7.8 ± 0.1**
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.0	4.5 ± 0.0*	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.1
Day 22	4.6 ± 0.1	4.7 ± 0.0	4.7 ± 0.1	4.8 ± 0.1**	4.8 ± 0.1*	4.9 ± 0.1**
Week 14	5.1 ± 0.2	5.1 ± 0.1	5.3 ± 0.1*	5.4 ± 0.1**	5.4 ± 0.1**	5.7 ± 0.1**
Bile acids (µmol/L)						
Day 4	26.4 ± 1.3	24.6 ± 0.8	27.3 ± 2.2	26.9 ± 1.6	40.3 ± 3.8**	31.7 ± 2.8*
Day 22	24.0 ± 0.8	30.1 ± 1.5*	27.0 ± 1.4	23.1 ± 1.1	29.1 ± 1.7	27.2 ± 1.0
Week 14	27.0 ± 1.7	34.3 ± 2.9	32.6 ± 1.4	34.8 ± 3.3	30.8 ± 1.5	36.4 ± 3.2*

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

The absolute and relative (except 100 ppm) right kidney weights of all exposed groups of males and of female groups exposed to 30 ppm or greater were significantly greater than those of the control groups (Table 6 and Table C-2). Absolute and relative liver weights of males exposed to 100 ppm or greater and females exposed to 30 ppm or greater were significantly greater than those of the controls.

Epididymal sperm counts (total and sperm/mg cauda) were significantly lower in males exposed to 1,000 ppm (Table 7 and Table D-1). There were no test article-related changes in the estrous cyclicity of rats exposed to *o*-chloropyridine (Table D-2, Table D-3, and Figure D-1).

Table 6. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
n	1	10	10	10	10	10
Male						
Necropsy body wt	320 ± 6	326 ± 7	326 ± 8	326 ± 8	309 ± 7	268 ± 6**
R. Kidney						
Absolute	0.95 ± 0.01	1.02 ± 0.02*	1.04 ± 0.03*	1.01 ± 0.02*	1.02 ± 0.03*	1.07 ± 0.02**
Relative	2.978 ± 0.065	3.144 ± 0.036*	3.172 ± 0.052*	3.110 ± 0.081	3.304 ± 0.030**	4.012 ± 0.052**
Liver						
Absolute	10.91 ± 0.29	11.76 ± 0.29	10.87 ± 0.78	13.17 ± 0.35**	13.51 ± 0.40**	14.33 ± 0.36**
Relative	34.101 ± 0.737	36.114 ± 0.615	33.092 ± 1.788	40.530 ± 1.053**	43.672 ± 0.620**	53.553 ± 0.653**
Female						
Necropsy body wt	195 ± 4	200 ± 2	200 ± 4	196 ± 2	190 ± 3	174 ± 2**
R. Kidney						
Absolute	0.65 ± 0.02	0.67 ± 0.01	0.70 ± 0.01*	0.69 ± 0.02*	0.71 ± 0.01**	0.74 ± 0.01**
Relative	3.317 ± 0.047	3.347 ± 0.032	3.472 ± 0.050*	3.530 ± 0.051**	3.714 ± 0.058**	4.259 ± 0.040**
Liver						
Absolute	5.80 ± 0.22	6.14 ± 0.14	6.77 ± 0.17**	6.89 ± 0.12**	7.94 ± 0.34**	9.06 ± 0.21**
Relative	29.632 ± 0.735	30.698 ± 0.619	33.784 ± 0.410**	35.152 ± 0.373**	41.684 ± 1.375**	52.086 ± 0.597**

*Significantly different ($P \leq 0.05$) from the control group by Williams' 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).

Table 7. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	100 ppm	300 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	320 ± 6	326 ± 8	309 ± 7	268 ± 6**
L. Cauda epididymis	0.1510 ± 0.0067	0.1563 ± 0.0040	0.1548 ± 0.0041	0.1503 ± 0.0037
L. Epididymis	0.4239 ± 0.0177	0.4579 ± 0.0097	0.4457 ± 0.0063	0.4222 ± 0.0079
L. Testis	1.4547 ± 0.0387	1.5359 ± 0.0227	1.4795 ± 0.0269	1.4671 ± 0.0212
Spermatid measurements				
Spermatid heads (10^6/testis)	169.75 ± 7.20	182.13 ± 5.88	170.88 ± 4.14	166.75 ± 4.25
Spermatid heads (10^6/g testis)	129.7 ± 3.6	131.9 ± 4.6	126.8 ± 2.8	125.6 ± 3.0
Epididymal spermatozoal measurements				
Sperm motility (%)	77.6 ± 1.0	71.7 ± 8.0	76.9 ± 1.4	77.4 ± 1.0
Sperm (10^6/cauda epididymis)	60.00 ± 4.43	68.75 ± 6.84	62.15 ± 5.53	42.40 ± 2.02*
Sperm (10^6/g cauda epididymis)	402 ± 29	443 ± 46	400 ± 32	283 ± 13*

*Significantly different (P ≤ 0.05) from the control group by Shirley's or Dunn's test.
**Significantly different (P ≤ 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 (tissue weights) or Dunn's test (spermatid measurements and sperm motility).

Incidences of clear cell focus in the liver were significantly increased in male rats exposed to 1,000 ppm; a slight increase was also observed in females at 1,000 ppm (Table 8, Table A-1, and Table A-2). The incidences of hepatocyte cytoplasmic vacuolization were significantly increased in 300 and 1,000 ppm males and in 1,000 ppm females; the severity of this lesion was increased in 1,000 ppm males.

Clear cell foci were scattered throughout the section and were characterized by enlarged hepatocytes with clear cytoplasm, particularly in the perinuclear region, but often involving the entire cell. The increased size of the hepatocytes occasionally resulted in minimal compression at the margin of the lesion. Hepatocyte cytoplasmic vacuolization was characterized by individual or clusters of large hepatocytes with pale cytoplasm and indistinct or microvesicular cytoplasmic vacuoles scattered throughout the section.

Incidences of hematopoietic cell proliferation in the spleen were significantly increased in 300 and 1,000 ppm males and in 1,000 ppm females (Table 8, Table A-1, and Table A-2). Incidences of splenic congestion were significantly increased in 1,000 ppm males and in most exposed groups of females. Pigmentation occurred in the spleen of every rat in the study, but the severity of the lesion was increased in 300 and 1,000 ppm males and all exposed groups of females.

Table 8. Incidences of Selected Nonneoplastic Lesions in Rats in the Three-month Drinking Water Study of *o*-Chloropyridine

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
Liver[a]	10	10	10	10	10	10
Clear Cell Focus[b]	0	0	0	0	0	6**
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	0	6** (1.0)[c]	9** (1.8)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	4 (1.8)	8 (2.0)	6 (1.8)	6 (1.7)	10** (2.0)	10** (2.0)
Congestion	4 (1.0)	1 (1.0)	2 (1.0)	0*	7 (1.0)	10** (1.0)
Pigmentation	10 (1.0)	10 (1.0)	10 (1.3)	10 (1.0)	10 (2.0)	10 (2.0)
Bone Marrow	10	10	10	10	10	10
Hyperplasia	1 (1.0)	0	2 (1.0)	1 (1.0)	9** (1.0)	10** (1.9)
Kidney	10	10	10	10	10	10
Renal Tubule, Accumulation, Hyaline Droplet	10 (2.1)	10 (2.4)	10 (2.3)	9 (1.8)	5* (1.2)	0**
Female						
Liver	10	10	10	10	10	10
Clear Cell Focus	0	0	0	0	0	2
Hepatocyte, Vacuolization Cytoplasmic	0	0	0	0	0	9** (1.3)
Spleen	10	10	10	10	10	10
Hematopoietic Cell Proliferation	3 (2.0)	3 (1.3)	6 (1.5)	3 (1.7)	7 (1.7)	10** (1.9)
Congestion	1 (2.0)	7** (1.3)	5 (1.6)	6* (1.3)	6* (1.0)	10** (1.8)
Pigmentation	10 (2.0)	10 (2.2)	10 (2.3)	10 (2.4)	10 (2.2)	10 (3.0)
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	0	0	0	3 (1.0)	9** (1.1)

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

Splenic hematopoietic cell proliferation was characterized by an increase of primarily erythroid precursors in the sinusoids of the spleen above background levels. Severity grades were evaluated semiquantitatively and ranged from a minimal to mild increase in cellularity. Background hematopoietic cell presence in control animals was fairly minimal although all animals had at least some hematopoietic precursors in the spleen characterized by scattered individual and small clusters of hematopoietic cells throughout the red pulp. The control males had a slightly higher background level of hematopoietic cells than the control females and therefore the severity grade of hematopoietic cell proliferation was judged relative to the

appropriate sex-matched control. Minimal hematopoietic proliferation consisted of 10% to 20% increased numbers of small clusters of hematopoietic cells in the red pulp compared to the majority of the controls. Mild hematopoietic cell proliferation was characterized by an approximately 21% to 50% increase in numbers of small clusters throughout the red pulp, but without confluence of clusters of hematopoietic cells. As stated above, background hematopoiesis in controls was not particularly prominent and therefore a minimal to mild increase in hematopoietic cells still did not result in loss of architecture or impingement on the white pulp elements.

Splenic congestion was characterized by an increased amount of blood in the red pulp of the spleen compared to controls. Severity of congestion was assessed semiquantitatively by the numbers (density) of red cells in the red pulp and varied from minimal to mild. As the numbers of red cells increased, less space was noted around individual red blood cells and the overall color of the spleen became more brightly eosinophilic. Minimal congestion had approximately 10% to 20% more red blood cells than controls and was best appreciated in the subcapsular red pulp. Mild congestion had 21% to 35% more red cells in sinusoids than controls, particularly in the subcapsular areas at the lateral edges of the spleen as seen on cross section, and also in red pulp in the central areas of the spleen. However, with both minimal and mild congestion, the overall architecture of the spleen was retained.

Pigmentation in the spleen was characterized by an increase in gold-colored pigment within macrophages. It is suspected that this pigmentation represents hemosiderin. Severity of pigmentation was assessed semiquantitatively and a threshold was not utilized. All spleens had at least some pigmentation with increasing severity in both the number of macrophages that contained pigment and in the amount of pigment within the macrophages. Minimal pigmentation was characterized by a faint gold pigment in 20% to 40% of individual macrophages scattered throughout the red pulp. Mild pigmentation was characterized by an approximately 50% increase in the amount of pigment in individual macrophages so that the cytoplasm was a more intense gold color. Also, mild pigmentation was characterized by an increased number of pigment-containing macrophages with at least 50% of macrophages containing pigment; occasional clusters of macrophages with distended cytoplasm containing pigment were noted. Moderate pigmentation had increased numbers of macrophages containing pigment and greater than 80% of macrophages had at least some pigmented material in the cytoplasm. Clusters of macrophages, containing abundant pigment distending the cytoplasm, were more numerous in spleens with moderate pigmentation than in spleens with mild pigmentation.

The incidences of bone marrow hyperplasia were significantly increased in 300 and 1,000 ppm males and 1,000 ppm females (Table 8, Table A-1, and Table A-2). Bone marrow hyperplasia was evaluated for overall cellularity of hematopoietic cells (myeloid, erythroid, and megakaryocytic cell lines). The cellularity was assessed semiquantitatively as a percentage of hematopoietic cells to fat in the marrow of the femur. In control males, the bone marrow was approximately 20% hematopoietic cells and 80% fat. In males, minimal hyperplasia was characterized by 21% to 40% hematopoietic cells and mild hyperplasia was diagnosed when 41% to 60% of the marrow was composed of hematopoietic cells. Control females had a higher percentage of fat in the marrow than male controls with approximately 10% hematopoietic cells and 90% fat. Minimal hyperplasia was characterized by approximately 11% to 20% hematopoietic cells and 80% fat. Mild hyperplasia was diagnosed when the marrow contained approximately 21% to 50% hematopoietic cells. Bone marrow hyperplasia was characterized by

an increase in erythroid and myeloid cell lines with all maturation stages represented. Numbers of megakaryocytes also increased with increasing bone marrow cellularity, and mast cells tended to increase with increasing cellularity. The myeloid/erythroid ratio was maintained between 1:1 and 2.5:1 across all groups, although there was a slight relative increase in the erythroid cells.

Significant decreases in the incidences of renal tubule hyaline droplet accumulation in the kidney occurred in 300 and 1,000 ppm males (Table 8 and Table A-1). Hyaline droplets are normal lysosomal accumulations of α2u-globulin, a protein specific to male rats, from the glomerular filtrate. This eosinophilic accumulation normally occurs within the cytoplasm of proximal convoluted tubules in the renal cortex.

Mice

Two-week Dermal Study

All mice survived to the end of the study (Table 9). The final mean body weights and body weight gains of all dosed groups were similar to those of the vehicle control groups. There were no clinical findings related to *o*-chloropyridine administration.

Relative right kidney weights of 50 and 100 mg/kg males and 25 mg/kg females were significantly greater than those of the vehicle controls (Table 10 and Table C-3). No gross or microscopic lesions were considered related to *o*-chloropyridine administration.

Exposure concentration selection rationale: Exposure concentrations for the 3-month drinking water study were selected for comparison to the 3-month pyridine study results[33].

Table 9. Survival and Body Weights of Mice in the Two-week Dermal Study of *o*-Chloropyridine[a]

Dose (mg/kg)	Survival[b]	Initial Body Weight (g)	Final Body Weight (g)	Change in Body Weight (g)	Final Weight Relative to Controls (%)
Male					
0	5/5	23.7 ± 0.3	25.7 ± 0.3	2.0 ± 0.3	
6.25	5/5	23.3 ± 0.7	26.4 ± 0.5	3.1 ± 0.4	103
12.5	5/5	23.5 ± 0.6	26.8 ± 0.4	3.3 ± 0.6	104
25	5/5	23.6 ± 0.7	26.1 ± 0.5	2.5 ± 0.4	102
50	5/5	23.5 ± 0.5	25.7 ± 0.3	2.2 ± 0.2	100
100	5/5	23.1 ± 0.9	25.6 ± 0.7	2.5 ± 0.3	100
Female					
0	5/5	19.9 ± 0.7	23.2 ± 0.6	3.3 ± 0.2	
6.25	5/5	19.5 ± 0.6	23.1 ± 0.4	3.6 ± 0.3	100
12.5	5/5	19.8 ± 0.6	22.8 ± 0.6	3.0 ± 0.3	98
25	5/5	19.8 ± 0.6	23.0 ± 0.6	3.2 ± 0.2	99
50	5/5	19.9 ± 0.7	22.7 ± 0.6	2.8 ± 0.4	98
100	5/5	20.0 ± 0.4	23.3 ± 0.4	3.3 ± 0.2	100

[a]Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.
[b]Number of animals surviving at 17 days/number initially in group.

Table 10. Kidney Weights and Kidney-Weight-to-Body-Weight Ratios for Mice in the Two-week Dermal Study of *o*-Chloropyridine[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.7 ± 0.3	26.4 ± 0.5	26.8 ± 0.4	26.1 ± 0.5	25.7 ± 0.3	25.6 ± 0.7
R. Kidney						
Absolute	0.26 ± 0.00	0.27 ± 0.01	0.28 ± 0.00	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
Relative	10.059 ± 0.254	10.379 ± 0.134	10.608 ± 0.230	10.544 ± 0.316	10.940 ± 0.277*	10.921 ± 0.248*
Female						
Necropsy body wt	23.2 ± 0.6	23.1 ± 0.4	22.8 ± 0.6	23.0 ± 0.6	22.7 ± 0.6	23.3 ± 0.4
R. Kidney						
Absolute	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
Relative	8.383 ± 0.185	8.835 ± 0.119	8.598 ± 0.185	9.074 ± 0.133*	8.613 ± 0.194	8.849 ± 0.172

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

Three-month Drinking Water Study

There were no treatment-related deaths in either sex (one control female was found dead) (Table 11). The final mean body weight and body weight gain of 1,000 ppm males were significantly less than those of the control group; the mean body weight gain of 300 ppm females was significantly greater than that of the controls (Table 11 and Figure 3). Water consumption by the 1,000 ppm groups was less than that of the control groups during the first week of the study. Drinking water concentrations of 10, 30, 100, 300, and 1,000 ppm resulted in average daily doses of approximately 1.5, 4.5, 15, 41, and 110 mg *o*-chloropyridine/kg body weight to males and 1.3, 4, 12, 38, and 92 mg/kg to females. There were no clinical findings related to *o*-chloropyridine exposure.

Table 11. Survival, Body Weights, and Water Consumption of Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

Concentration (ppm)	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 14
Male							
0	10/10	23.2 ± 0.3	40.8 ± 0.9	17.6 ± 0.8		4.8	4.5
10	10/10	23.2 ± 0.4	42.4 ± 1.1	19.1 ± 1.2	104	5.1	4.6
30	10/10	23.3 ± 0.4	42.4 ± 0.6	19.2 ± 0.6	104	5.4	4.9
100	10/10	23.3 ± 0.3	40.2 ± 1.2	16.8 ± 1.0	99	5.3	5.4
300	10/10	23.1 ± 0.3	40.5 ± 1.2	17.4 ± 1.1	99	4.4	4.7
1,000	10/10	23.1 ± 0.2	33.0 ± 0.5**	9.8 ± 0.6**	81	3.2	3.3

Concentration (ppm)	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 14
Female							
0	9/10[c]	18.6 ± 0.4	28.5 ± 1.4	9.7 ± 1.2		2.8	3.5
10	10/10	19.1 ± 0.3	31.8 ± 1.0	12.8 ± 1.0	112	3.0	3.3
30	10/10	18.8 ± 0.3	31.2 ± 1.3	12.4 ± 1.1	109	2.7	3.4
100	10/10	19.0 ± 0.3	30.3 ± 1.1	11.3 ± 0.9	106	2.3	3.4
300	10/10	18.1 ± 0.5	31.9 ± 0.8	13.8 ± 0.7*	112	3.3	3.4
1,000	10/10	18.6 ± 0.4	28.4 ± 0.6	9.9 ± 0.5	100	1.6	2.8

*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]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.
[c]Week of death: 11.

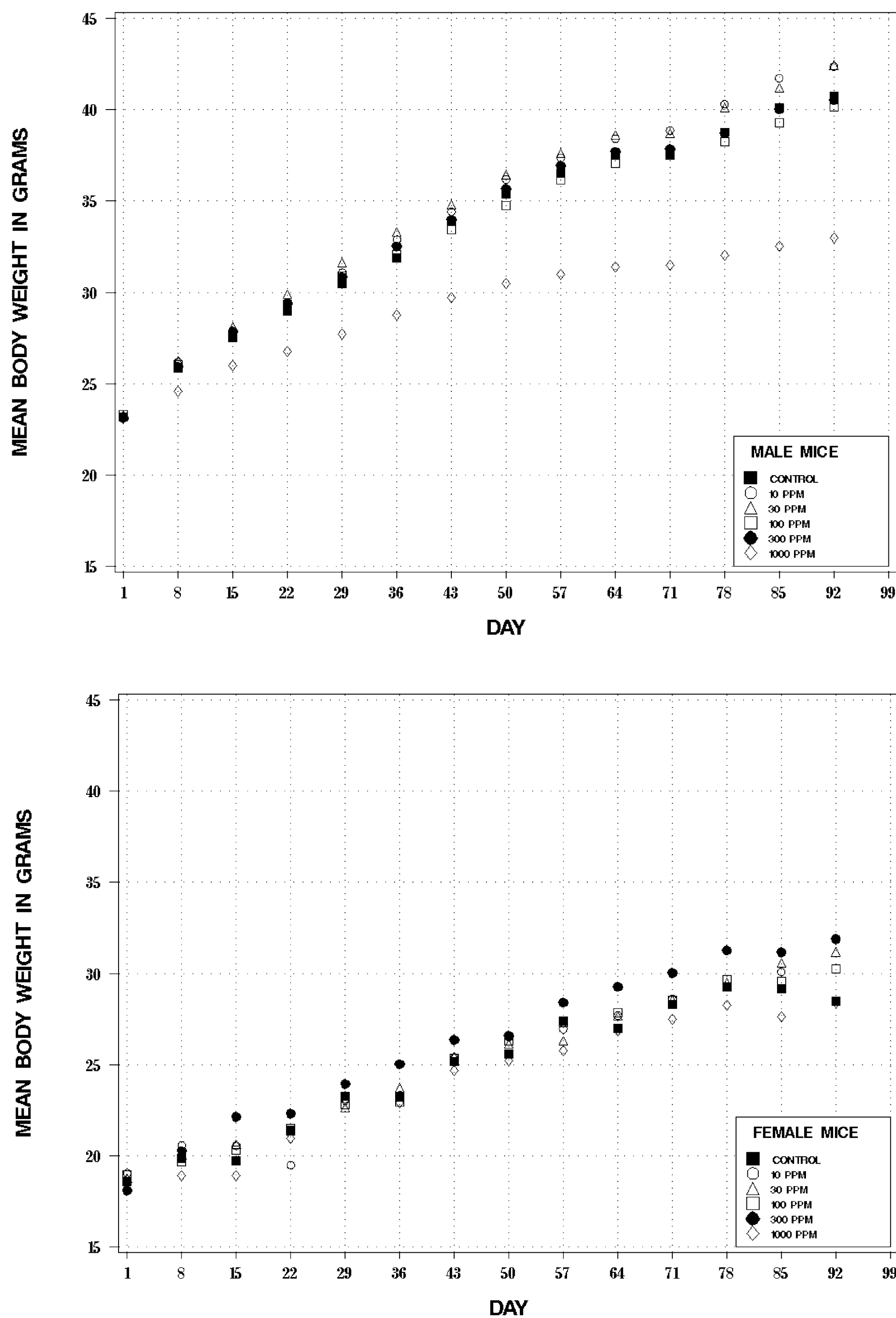

Figure 3. Growth Curves for Mice Exposed to *o*-Chloropyridine in Drinking Water for Three Months

The hematology data for mice are presented in Table 12 and Table B-2. Similar to what occurred in the rat study, at week 14, a decreased erythron occurred; however, this was only observed in the 1,000 ppm females. The erythron decrease was small (<10%) and was demonstrated by decreases in hematocrit value, hemoglobin concentration and erythrocyte count. As with the rats,

the erythron decrease was accompanied by a small reduction (approximately 3%) in erythrocyte size, evidenced by a decrease in the mean cell volume.

The absolute and relative liver weights of all exposed groups of males and of 300 (absolute, 32.8%) and 1,000 ppm (absolute, 59%) females were significantly greater than those of the control groups (Table 13 and Table C-4). The absolute (15.8%) and relative right kidney weights of 1,000 ppm females were significantly greater than those of the controls. There were no significant differences in any of the reproductive organ weights or sperm parameters of males, or in the estrous cyclicity of females, at any exposure concentration tested when compared to the control groups (Table D-4, Table D-5, and Table D-6 and Figure D-2).

Table 12. Selected Hematology Data for Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Hematocrit (%)	51.2 ± 0.4	51.4 ± 0.5	50.4 ± 0.7	50.2 ± 0.4	50.3 ± 0.6	50.5 ± 0.6
Hemoglobin (g/dL)	16.4 ± 0.1	16.5 ± 0.1	16.1 ± 0.2	16.1 ± 0.1	16.2 ± 0.2	16.2 ± 0.2
Erythrocytes (10^6/µL)	10.63 ± 0.09	10.78 ± 0.10	10.46 ± 0.13	10.65 ± 0.07	10.67 ± 0.11	10.69 ± 0.08
Reticulocytes (10^6/µL)	0.25 ± 0.02	0.27 ± 0.02	0.29 ± 0.03	0.28 ± 0.02	0.28 ± 0.03	0.26 ± 0.03
Mean cell volume (fL)	47.3 ± 0.3	47.3 ± 0.3	47.5 ± 0.2	46.8 ± 0.1	47.0 ± 0.4	46.7 ± 0.2
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.2	32.1 ± 0.1	32.0 ± 0.1	32.2 ± 0.1	32.2 ± 0.1	32.1 ± 0.1
Female						
n	9	10	10	10	10	10
Hematocrit (%)	50.9 ± 1.1	50.5 ± 0.7	48.9 ± 0.4	50.5 ± 0.4	48.8 ± 0.6	46.6 ± 0.4**
Hemoglobin (g/dL)	16.2 ± 0.3	16.0 ± 0.2	15.7 ± 0.1	16.2 ± 0.1	15.7 ± 0.2	15.1 ± 0.1**
Erythrocytes (10^6/µL)	10.56 ± 0.20	10.42 ± 0.12	10.23 ± 0.09	10.54 ± 0.07	10.22 ± 0.13	9.96 ± 0.09**
Reticulocytes (10^6/µL)	0.40 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.31 ± 0.03
Mean cell volume (fL)	48.2 ± 0.2	48.2 ± 0.1	47.9 ± 0.2	47.9 ± 0.2	47.7 ± 0.2	46.9 ± 0.2**
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.3 ± 0.0	15.3 ± 0.1	15.4 ± 0.1	15.4 ± 0.0	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.8 ± 0.1	31.7 ± 0.1	32.0 ± 0.1	32.0 ± 0.1	32.2 ± 0.1	32.4 ± 0.1**

**Significantly different ($P \leq 0.01$) from the control group by Shirley's test.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

Table 13. Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	40.8 ± 0.9	42.4 ± 1.1	42.4 ± 0.6	40.2 ± 1.2	40.5 ± 1.2	33.0 ± 0.5**
R. Kidney						
Absolute	0.34 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.31 ± 0.01
Relative	8.237 ± 0.218	8.203 ± 0.090	8.193 ± 0.244	8.631 ± 0.217	8.556 ± 0.229	9.242 ± 0.369*
Liver						
Absolute	1.70 ± 0.06	1.89 ± 0.07*	1.95 ± 0.04*	1.84 ± 0.07*	2.01 ± 0.08**	1.95 ± 0.05**
Relative	41.624 ± 0.778	44.531 ± 0.718*	45.851 ± 0.819**	45.766 ± 0.974**	49.467 ± 0.796**	59.121 ± 0.830**
Female						
n	9	10	10	10	10	10
Necropsy body wt	28.5 ± 1.4	31.8 ± 1.0	31.2 ± 1.3	30.3 ± 1.1	31.9 ± 0.8	28.4 ± 0.6
R. Kidney						
Absolute	0.19 ± 0.01	0.20 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.00	0.22 ± 0.01**
Relative	6.866 ± 0.352	6.203 ± 0.206	6.212 ± 0.170	6.232 ± 0.174	6.321 ± 0.154	7.676 ± 0.156*
Liver						
Absolute	1.22 ± 0.09	1.37 ± 0.04	1.34 ± 0.05	1.35 ± 0.07	1.62 ± 0.05**	1.94 ± 0.0.05**
Relative	42.778 ± 1.560	43.065 ± 1.066	43.266 ± 0.645	44.466 ± 1.122	50.818 ± 1.290**	68.154 ± 1.026**

*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).

Incidences of hepatocyte centrilobular hypertrophy occurred with positive trends in exposed groups of males and females, and the incidences were significantly increased in the 300 and 1,000 ppm groups compared to those in the controls (Table 14, Table A-3, and Table A-4); the severity of this lesion also increased with exposure concentration. The lesion was characterized by minimal to mild enlargement (hypertrophy) of hepatocytes that were centrilobular in distribution. Hypertrophic hepatocytes had homogeneous or slightly granular eosinophilic cytoplasm. The nuclei were often enlarged and contained prominent basophilic chromatin aggregates.

Table 14. Incidences of Hepatocyte Centrilobular Hypertrophy in the Liver of Mice in the Three-month Drinking Water Study of *o*-Chloropyridine

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
Number Examined Microscopically	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy[a]	0	0	0	1 (1.0)[b]	6** (1.5)	9** (2.1)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Centrilobular, Hepatocyte, Hypertrophy	0	0	0	0	4* (1.0)	10** (1.5)

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

Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

The metabolism and disposition of *o*-chloropyridine was investigated following a single oral, intravenous, or intraperitoneal dose in male F344 rats (Appendix J). For all studies, formulations were prepared by addition of Alkamuls® EL-620/L (no more than 15% of the dose) (Rhodia, Inc., Cranbury, NJ) to known amounts of *o*-chloropyridine following which distilled deionized water and the appropriate amount of [^{14}C]-*o*-chloropyridine were added. Dosing volumes were either 5 mL/kg (oral gavage and intraperitoneal injection) or 1 mL/kg (intravenous injection). Oral doses were administered by intragastric gavage using a syringe equipped with a 16-gauge gavage needle. Intravenous doses were injected into a lateral tail vein using a syringe equipped with a 27-gauge needle.

Following gavage administration of 0.1 or 10 mg [^{14}C]-*o*-chloropyridine/kg, there was no dose-related difference in the disposition. Approximately 82% of the administered dose was recovered by 72 hours following administration. The absorption was rapid with maximum blood concentration being reached within 30 minutes following administration. The distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half-lives, estimated based on the measurement of the total radioactivity in blood were 1.14 hours and 46 hours, respectively. Excretion in urine accounted for approximately 36% to 39% and that in feces about 28%. The observation that 27% of a 50 mg/kg intraperitoneal dose was excreted in bile in rats by 4 hours after administration suggests that the high fecal excretion following gavage administration was likely due to biliary excretion and not due to poor absorption. Approximately 11% to 13% of the gavage dose was recovered as CO_2 and approximately 1% was eliminated in breath as volatile organics. The total radioactivity in tissue at 72 hours following administration was approximately 3% to 4% with the highest tissue:blood ratios observed in the liver and kidney. Profiling of urine showed that the compound is readily metabolized. Three major metabolites carrying most of the radioactivity were found to contain disubstituted pyridine structures, one of which was identified as 2-chloro-5-hydroxypyridine.

Following intravenous administration of 1 mg/kg [^{14}C]-*o*-chloropyridine in male F344/N rats, the distribution ($T_{1/2\alpha}$) and elimination ($T_{1/2\beta}$) half-lives were 0.103 hours and 1.04 hours, respectively, based on the measurement of parent compound (Appendix J).

Genetic Toxicology

o-Chloropyridine (10 to10,000 µg/plate) was mutagenic in *Salmonella typhimurium* strains TA98 and TA100 when tested with induced rat or hamster liver S9 metabolic activation enzymes; no mutagenic activity was observed in either strain in the absence of metabolic activation (Table E-1). In both TA98 and TA100, the mutagenic activity observed with *o*-chloropyridine was stronger with hamster S9 (higher magnitude response observed at lower concentrations of *o*-chloropyridine). In vivo, no increases in the frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male or female mice exposed to *o*-chloropyridine (10 to 1,000 ppm) in drinking water for 3 months (Table E-2). In addition, no significant exposure concentration-related changes in the percentage of immature (polychromatic) erythrocytes were observed, suggesting that exposure to *o*-chloropyridine did not alter the process of erythropoiesis in the bone marrow of mice.

Discussion

o-Chloropyridine is used as a key intermediate for the manufacturing of pyrithione-based chemicals used in cosmetics and agriculture. It is also a starting material in the production of certain antihistamines and antiarrhythmic pharmaceuticals. In 2006, the production and import volume of *o*-chloropyridine in the United States was reported to range from 1 million to 10 million pounds[11]. Occupational exposure can occur during the synthesis of *o*-chloropyridine and during its use as an industrial chemical intermediate. High vapor concentrations due to liquid spills have been detected in some industrial processing areas, suggesting the potential for significant exposure[17]. Environmental exposure to *o*-chloropyridine is also possible; it has been found as a trace organic contaminant in process streams and wastewater as well as some drinking water sources[20; 21]. Despite the high production volume and potential for occupational and environmental exposure, there is little toxicity data available for this compound and no guidelines have been set by the National Institute for Occupational Safety and Health or the Occupational Safety and Health Administration. The current report describes the 2-week dermal and 3-month drinking water studies for *o*-chloropyridine administered to F344/N rats and B6C3F1/N mice. For the 3-month studies, the route of exposure was changed to drinking water, allowing for comparison of *o*-chloropyridine toxicity with the results of the NTP 3-month drinking water study of a structurally similar compound, pyridine[33]. Toxicity targets of *o*-chloropyridine were similar across species and routes of exposure, including increased liver weight with accompanying histologic changes in the liver for the 3-month studies.

In the 2-week dermal studies, all rats and mice survived until the end of the study with no significant changes in body weights. Some significant changes in liver and kidney weights were observed, with no supporting histologic changes.

In the 3-month drinking water studies with *o*-chloropyridine, there was no effect on survival in rats or mice. The major findings in rats related to a possible inefficient erythropoiesis, evidenced by changes in erythron, bone marrow, and spleen. In mice, the major finding was greater liver weights, compared to controls, accompanied by centrilobular hepatocyte hypertrophy.

In the current 3-month drinking water study, significant changes, both increases and decreases, in hematology values were observed in male rats. On day 4, hematology results demonstrated an increase in circulating erythron (indicated by hematocrit, hemoglobin concentration, and erythrocytes) in 1,000 ppm male rats. This change was likely a physiologic response secondary to dehydration, due to a 50% decrease in water consumption. In addition, no changes in renal function were observed, indicated by creatinine concentration, supporting a dehydration hypothesis. Observations of exposure concentration-related and progressive hyperalbuminemia and hyperproteinemia should be considered a physiological response to dehydration and are unlikely to be a toxicologic response to *o*-chloropyridine.

While early changes in erythron were likely due to dehydration, by day 22, changes in hematocrit, hemoglobin, and erythrocytes were reversed, demonstrating a decrease in erythron that persisted until the end of the study. At week 14, an increase in reticulocyte count was also observed; these results together indicate an erythropoietic response that is further supported by increased incidences of hematopoietic cell proliferation and congestion in the spleen and hyperplasia of the bone marrow. The minimal microcytic, normochromic, and responsive

erythron decreases observed on day 22 and at week 14 involved production of smaller erythrocytes and would indicate some mild exposure-related ineffective erythropoiesis potentially involving an alteration in iron availability/metabolism or the production or availability of heme or hemoglobin. Classically, microcytic erythron decreases have been associated with an iron deficiency[49]. Because there was no clinical evidence of hemorrhage or blood loss, however, the microcytic change would suggest an ineffective erythropoiesis related to some alteration in iron and/or heme/hemoglobin metabolism. Such microcytic-type erythron decreases have been observed in rats with multiple agent types [e.g., lead[50]; cupric sulfate[51]; estragole[52]]. In fact, in the 3-month study of pyridine, a similar iron deficiency-like response occurred in rats[33]. Mice demonstrated a similar decrease in erythron, although they appeared to be less sensitive, with effects seen only in high-dose females and without an observable erythropoietic response.

A decrease in the incidence and severity of hyaline droplet accumulation was observed in male rats in the 300 and 1,000 ppm groups. Hyaline droplets are normal lysosomal accumulations of α2u-globulin, a protein specific to male rats that is synthesized in the liver. Decreased synthesis of this protein by the liver may explain the decreased hyaline droplet accumulation that was observed. A study in rats treated with diethylnitrosamine reported a decrease in α2u-globulin mRNA in the liver, demonstrating that some chemicals are capable of affecting α2u-globulin synthesis[53]. In the present study, α2u-globulin mRNA was not measured, however decreased synthesis of the α2u-globulin protein is a potential explanation for the lower hyaline droplet accumulation that was observed in male rat kidney.

In humans, it has been reported that the pathology associated with *o*-chloropyridine is similar to that of pyridine exposure. Acute, low-dose exposures, that do not cause clinical symptoms, have been associated with centrilobular fatty degeneration, congestion, and cellular infiltration in the liver; low-dose chronic exposures may lead to cirrhosis[4]. In the current study, *o*-chloropyridine exposure in rats resulted in histopathologic lesions of the liver that may indicate a metabolic, adaptive response to treatment, including clear cell foci and hepatocyte cytoplasmic vacuolization. These changes were also observed in the 2-week dermal study with *o*-chloropyridine. While the liver was also a target in the pyridine 3-month drinking water study[33], the lesions produced in the F344/N rat were of a different nature, suggesting direct toxicity (centrilobular degeneration, hypertrophy, inflammation, and pigmentation). In addition to pyridine, NTP also performed a 3-month drinking water study in F344/N rats with a structurally similar compound, β-picoline, which targeted the kidney, resulting in increased severity of α2u-globulin-mediated nephropathy and produced no liver lesions[54]. Large differences also exist in the bacterial mutagenicity of these three structurally similar compounds; *o*-chloropyridine is a potent bacterial mutagen while neither β-picoline nor pyridine is mutagenic.

Under the conditions of these 3-month drinking water studies, there were treatment-related organ weight changes and lesions in male and female rats and mice. The major target tissues in rats affected by *o*-chloropyridine exposure included the kidney, spleen, bone marrow, and liver; the major target organ in mice was the liver. The measurement most sensitive to *o*-chloropyridine exposure in male rats was increased absolute (all exposure groups) and relative (all exposure groups except 100 ppm) kidney weights in the absence of histopathologic changes [lowest-observed-effect level (LOEL) = 10 ppm]. In female rats, a LOEL of 10 ppm was based on splenic congestion, observed in all treated groups (except 30 ppm); with hematopoietic cell

proliferation and pigmentation in the spleen, bone marrow hyperplasia, and hematological changes at higher exposure concentrations. This pattern of erythropoietic responses in the spleen and bone marrow and hematologic changes was also observed in male rats at 300 ppm or greater. In male mice, absolute and relative liver weights were significantly higher than controls in all exposed groups (LOEL 10 ppm), with histologic changes (centrilobular hepatocyte hypertrophy) occurring at 300 and 1,000 ppm. In female mice, absolute and relative liver weights were significantly greater than controls, with increased incidences of centrilobular hepatocyte hypertrophy occurring at similar exposure concentrations (LOEL 300 ppm).

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Appendix A. Summary of Lesions in Rats and Mice

Tables

Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Drinking Water Study of *o*-Chloropyridine.. A-2
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Table A-1. Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Basophilic focus	0	0	0	0	0	2 (20%)
Clear cell focus	0	0	0	0	0	6 (60%)
Eosinophilic focus	0	0	0	0	0	1 (10%)
Hepatodiaphragmatic nodule	0	0	0	0	1 (10%)	0
Bile duct, hyperplasia	1 (10%)	0	0	0	0	0
Hepatocyte, vacuolization cytoplasmic	0	0	0	0	6 (60%)	9 (90%)
Salivary glands	(9)	(0)	(0)	(0)	(0)	(10)
Parotid gland, basophilic focus	2 (22%)	–	–	–	–	5 (50%)
Cardiovascular System						
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	4 (40%)	–	–	–	–	2 (20%)
Inflammation, chronic	1 (10%)	–	–	–	–	0
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Zona fasciculata, vacuolization cytoplasmic	9 (90%)	–	–	–	–	5 (50%)
Zona reticularis, vacuolization cytoplasmic	0	–	–	–	–	2 (20%)
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
Cyst	1 (10%)	–	–	–	–	0
Ectopic thymus	1 (10%)	–	–	–	–	1 (10%)
General Body System						
None	–	–	–	–	–	–
Genital System						
None	–	–	–	–	–	–
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia	1 (10%)	0	2 (20%)	1 (10%)	9 (90%)	6 (60%)

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(0)	(10)
Sinus, histiocytosis	0	–	–	–	–	2 (20%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion	4 (40%)	1 (10%)	2 (20%)	0	7 (70%)	10 (100%)
Hematopoietic cell proliferation	4 (40%)	8 (80%)	6 (60%)	6 (60%)	10(100%)	10(100%)
Pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Integumentary System						
None	–	–	–	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
Lung	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell, focal	1 (10%)	–	–	–	–	0
Metaplasia, osseous	2 (20%)	–	–	–	–	0
Alveolus, hemorrhage, focal	1 (10%)	–	–	–	–	1 (10%)
Special Senses System						
Eye	(10)	(0)	(0)	(0)	(0)	(10)
Posterior chamber, developmental malformation	1 (10%)	–	–	–	–	0
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization	0	1 (10%)	0	0	0	0
Nephropathy	3 (30%)	2 (20%)	5 (50%)	6 (60%)	3 (30%)	4 (40%)
Renal tubule, accumulation, hyaline droplet	10 (100%)	10 (100%)	10 (100%)	9 (90%)	5 (50%)	0

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

Table A-2. Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Clear cell focus	0	0	0	0	0	2 (20%)
Hepatocyte, vacuolization cytoplasmic	0	0	0	0	0	9 (90%)
Pancreas	(10)	(0)	(0)	(0)	(0)	(10)
Duct, hyperplasia	1 (10%)	–	–	–	–	0
Salivary glands	(10)	(0)	(0)	(0)	(0)	(10)
Parotid gland, basophilic focus	3 (30%)	–	–	–	–	2 (20%)
Cardiovascular System						
Heart	(10)	(0)	(0)	(0)	(0)	(10)
Cardiomyopathy	1 (10%)	–	–	–	–	0
Epicardium, inflammation	0	–	–	–	–	1 (10%)
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell, focal	0	–	–	–	–	1 (10%)
Pituitary gland	(9)	(0)	(0)	(0)	(0)	(10)
Pars distalis, cyst	1 (11%)	–	–	–	–	0
Thyroid gland	(10)	(0)	(0)	(0)	(0)	(10)
Cyst	2 (20%)	–	–	–	–	1 (10%)
Ectopic thymus	1 (10%)	–	–	–	–	0
General Body System						
None	–	–	–	–	–	–
Genital System						
Uterus	(10)	(0)	(0)	(0)	(0)	(10)
Dilatation	1 (10%)	–	–	–	–	3 (30%)
Hydrometra	1 (10%)	–	–	–	–	2 (20%)
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia	0	0	0	0	3 (30%)	9 (90%)

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Lymph node, mesenteric	(10)	(0)	(0)	(0)	(0)	(10)
Sinus, histiocytosis	6 (60%)	–	–	–	–	4 (40%)
Spleen	(10)	(10)	(10)	(10)	(10)	(10)
Congestion	1 (10%)	7 (70%)	5 (50%)	6 (60%)	6 (60%)	10 (100%)
Hematopoietic cell proliferation	3 (30%)	3 (30%)	6 (60%)	3 (30%)	7 (70%)	10 (100%)
Pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Thymus	(10)	(0)	(0)	(0)	(0)	(10)
Thymocyte, atrophy	1 (10%)	–	–	–	–	0
Integumentary System						
None	–	–	–	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
Lung	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular, mononuclear cell, focal	4 (40%)	–	–	–	–	3 (30%)
Alveolus, hemorrhage, focal	1 (10%)	–	–	–	–	0
Special Senses System						
Eye	(10)	(0)	(0)	(0)	(0)	(10)
Cornea, inflammation	0	–	–	–	–	1 (10%)
Sclera, inflammation	0	–	–	–	–	2 (20%)
Harderian gland	(10)	(0)	(0)	(0)	(0)	(10)
Infiltration cellular developmental malformation	0	–	–	–	–	1 (10%)
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization	0	0	1 (10%)	0	0	0
Nephropathy	2 (20%)	0	1 (10%)	1 (10%)	2 (20%)	5 (50%)

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

Table A-3. Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Male Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mononuclear cell, focal	1 (10%)	0	1 (10%)	0	1 (10%)	1 (10%)
Necrosis, focal	1 (10%)	0	2 (20%)	0	0	2 (20%)
Centrilobular, hepatocyte, hypertrophy	0	0	0	1 (10%)	6 (60%)	9 (90%)
Stomach, glandular	(10)	(0)	(0)	(0)	(0)	(10)
Cyst	0	–	–	–	–	1 (10%)
Cyst epithelial inclusion	2 (20%)	–	–	–	–	0
Cardiovascular System						
None	–	–	–	–	–	–
Endocrine System						
Adrenal cortex	(9)	(0)	(0)	(0)	(0)	(10)
Subcapsular, hyperplasia focal	0	–	–	–	–	2 (20%)
Parathyroid gland	(7)	(0)	(0)	(0)	(0)	(7)
Cyst	1 (14%)	–	–	–	–	0
General Body System						
None	–	–	–	–	–	–
Genital System						
None	–	–	–	–	–	–
Hematopoietic System						
Spleen	(10)	(0)	(0)	(0)	(0)	(10)
Hematopoietic cell proliferation	10 (100%)	–	–	–	–	10 (100%)
Integumentary System						
None	–	–	–	–	–	–
Musculoskeletal System						
None	–	–	–	–	–	–

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Nervous System						
None	–	–	–	–	–	–
Respiratory System						
None	–	–	–	–	–	–
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Mineralization	0	0	0	0	2 (20%)	0
Nephropathy	0	0	0	0	0	1 (10%)
Interstitium, infiltration cellular, mononuclear cell	0	0	1 (10%)	0	0	1 (10%)
Renal tubule, adenoma	0	0	1 (10%)	0	0	0
Renal tubule, casts protein	0	0	0	1 (10%)	0	0
Renal tubule, vacuolization cytoplasmic	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)

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

Table A-4. Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Female Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Natural death	1	–	–	–	–	–
Survivors						
Terminal sacrifice	9	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mononuclear cell, focal	0	1 (10%)	1 (10%)	0	1 (10%)	0
Necrosis, focal	0	0	0	0	0	1 (10%)
Centrilobular, hepatocyte, hypertrophy	0	0	0	0	4 (40%)	10 (100%)
Cardiovascular System						
None	–	–	–	–	–	–
Endocrine System						
Adrenal cortex	(10)	(0)	(0)	(0)	(0)	(10)
Subcapsular, hyperplasia, focal	10 (100%)	–	–	–	–	10 (100%)
General Body System						
None	–	–	–	–	–	–
Genital System						
Ovary	(10)	(0)	(0)	(0)	(0)	(10)
Teratoma, benign	1 (10%)	–	–	–	–	0
Uterus	(10)	(0)	(0)	(0)	(0)	(10)
Deciduoma, benign	0	–	–	–	–	1 (10%)
Endometrium, hyperplasia cystic	4 (40%)	–	–	–	–	3 (30%)
Hematopoietic System						
Spleen	(9)	(0)	(0)	(0)	(0)	(10)
Hematopoietic cell proliferation	9 (100%)	–	–	–	–	10 (100%)
Integumentary System						
Skin	(10)	(0)	(0)	(0)	(0)	(10)

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Dermis, inflammation	1 (10%)	–	–	–	–	0
Musculoskeletal System						
None	–	–	–	–	–	–
Nervous System						
None	–	–	–	–	–	–
Respiratory System	(10)	(0)	(0)	(0)	(0)	(10)
Lung						
Hemorrhage, focal	0	–	–	–	–	1 (10%)
Special Senses System						
None	–	–	–	–	–	–
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, mononuclear cell	0	0	1 (10%)	0	0	0
Mineralization	0	0	1 (10%)	0	0	0
Nephropathy	0	2 (20%)	0	0	0	0
Interstitium, infiltration cellular, mononuclear cell	1 (10%)	0	0	0	0	0

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

Appendix B. Clinical Pathology Results

Tables

Table B-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Drinking Water Study of *o*-Chloropyridine B-2
Table B-2. Hematology Data for Mice in the Three-month Drinking Water Study of *o*-Chloropyridine B-8

Table B-1. Hematology and Clinical Chemistry Data for Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
Hematology						
n						
Day 4	10	10	10	9	9	10
Day 22	10	10	10	10	10	10
Week 14	9	10	10	10	10	10
Hematocrit (%)						
Day 4	40.0 ± 0.4	39.8 ± 0.4	40.5 ± 0.6	38.8 ± 0.5	39.6 ± 0.5	43.3 ± 0.9*
Day 22	43.4 ± 0.4	42.7 ± 0.4	42.6 ± 0.4	41.2 ± 0.6**	41.5 ± 0.5**	39.6 ± 0.4**
Week 14	45.5 ± 0.6	45.2 ± 0.3	46.3 ± 0.3	44.5 ± 0.9	42.9 ± 0.4**	41.7 ± 0.4**
Hemoglobin (g/dL)						
Day 4	13.4 ± 0.1	13.4 ± 0.1	13.6 ± 0.2	13.1 ± 0.2	13.3 ± 0.2	14.5 ± 0.3*
Day 22	14.8 ± 0.1	14.5 ± 0.1	14.5 ± 0.1	14.1 ± 0.2**	14.1 ± 0.2**	13.5 ± 0.1**
Week 14	15.2 ± 0.2	15.1 ± 0.1	15.4 ± 0.1	15.0 ± 0.3	14.4 ± 0.1**	13.9 ± 0.1**
Erythrocytes (10^6/µL)						
Day 4	6.80 ± 0.08	6.85 ± 0.10	6.92 ± 0.10	6.72 ± 0.09	6.95 ± 0.10	7.53 ± 0.14**
Day 22	7.50 ± 0.07	7.35 ± 0.09	7.37 ± 0.08	7.18 ± 0.11*	7.30 ± 0.08	7.25 ± 0.08
Week 14	8.91 ± 0.11	8.75 ± 0.07	9.04 ± 0.08	8.76 ± 0.15	8.51 ± 0.06**	8.42 ± 0.09**
Reticulocytes (10^6/µL)						
Day 4	0.46 ± 0.04	0.45 ± 0.04	0.54 ± 0.02	0.50 ± 0.05	0.46 ± 0.03	0.39 ± 0.02
Day 22	0.33 ± 0.04	0.33 ± 0.02	0.28 ± 0.02	0.30 ± 0.02	0.36 ± 0.04	0.41 ± 0.02
Week 14	0.26 ± 0.01	0.27 ± 0.02	0.27 ± 0.02	0.25 ± 0.02	0.37 ± 0.02**	0.32 ± 0.03*
Mean cell volume (fL)						
Day 4	58.8 ± 0.3	58.1 ± 0.2	58.5 ± 0.3	57.8 ± 0.3*	56.9 ± 0.2**	57.5 ± 0.2**
Day 22	57.9 ± 0.3	58.0 ± 0.3	57.7 ± 0.2	57.3 ± 0.3	56.8 ± 0.3**	54.7 ± 0.2**
Week 14	51.2 ± 0.2	51.6 ± 0.2	51.3 ± 0.2	50.8 ± 0.3	50.5 ± 0.2*	49.3 ± 0.3**
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.1	19.5 ± 0.1	19.6 ± 0.1	19.5 ± 0.1	19.1 ± 0.1**	19.3 ± 0.1**
Day 22	19.8 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.4 ± 0.0**	18.7 ± 0.1**
Week 14	17.0 ± 0.0	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	16.9 ± 0.1	16.5 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.6 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.7 ± 0.1	33.6 ± 0.1	33.5 ± 0.1
Day 22	34.1 ± 0.1	34.1 ± 0.1	34.0 ± 0.1	34.2 ± 0.1	34.1 ± 0.1	34.1 ± 0.1
Week 14	33.3 ± 0.1	33.3 ± 0.1	33.3 ± 0.1	33.6 ± 0.1	33.5 ± 0.1	33.4 ± 0.1
Platelets (10^3/µL)						
Day 4	640.9 ± 8.6	625.4 ± 25.0	613.4 ± 26.7	606.3 ± 18.3	605.2 ± 28.6	608.0 ± 29.7
Day 22	527.2 ± 21.0	518.5 ± 21.1	586.0 ± 19.3	550.9 ± 18.4	542.0 ± 22.3	501.9 ± 22.7
Week 14	418.8 ± 34.9	443.3 ± 20.3	501.6 ± 13.6	419.0 ± 29.3	485.4 ± 23.6	503.6 ± 15.8

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Leukocytes (10^3/µL)						
Day 4	9.40 ± 0.21	9.02 ± 0.25	10.36 ± 0.29	9.82 ± 0.50	8.56 ± 0.36	11.62 ± 0.37**
Day 22	11.68 ± 0.43	12.03 ± 0.27	11.85 ± 0.29	11.26 ± 0.65	11.83 ± 0.44	10.68 ± 0.44
Week 14	10.30 ± 0.82	11.75 ± 0.50	12.26 ± 0.55	10.05 ± 0.74	10.88 ± 0.38	11.10 ± 0.48
Segmented neutrophils (10^3/µL)						
Day 4	0.58 ± 0.05	0.57 ± 0.06	0.66 ± 0.04	0.59 ± 0.08	0.61 ± 0.08	0.62 ± 0.03
Day 22	0.61 ± 0.05	0.63 ± 0.03	0.62 ± 0.05	0.50 ± 0.04	0.62 ± 0.03	0.47 ± 0.03
Week 14	0.56 ± 0.06	0.76 ± 0.11	0.72 ± 0.05	0.56 ± 0.06	0.55 ± 0.04	0.49 ± 0.03
Lymphocytes (10^3/µL)						
Day 4	8.58 ± 0.21	8.23 ± 0.24	9.44 ± 0.27	8.96 ± 0.46	7.70 ± 0.32	10.71 ± 0.35**
Day 22	10.80 ± 0.37	11.11 ± 0.23	10.94 ± 0.24	10.53 ± 0.58	10.94 ± 0.43	9.98 ± 0.42
Week 14	9.43 ± 0.73	10.51 ± 0.41	11.17 ± 0.51*	9.17 ± 0.68	10.05 ± 0.33	10.33 ± 0.43
Monocytes (10^3/µL)						
Day 4	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.16 ± 0.02	0.12 ± 0.01	0.15 ± 0.01
Day 22	0.13 ± 0.02	0.13 ± 0.01	0.14 ± 0.02	0.10 ± 0.02	0.13 ± 0.01	0.12 ± 0.01
Week 14	0.18 ± 0.03	0.23 ± 0.04	0.20 ± 0.03	0.15 ± 0.02	0.16 ± 0.02	0.19 ± 0.03
Basophils (10^3/µL)						
Day 4	0.104 ± 0.007	0.087 ± 0.006	0.116 ± 0.013	0.096 ± 0.006	0.094 ± 0.008	0.129 ± 0.010
Day 22	0.134 ± 0.020	0.144 ± 0.017	0.132 ± 0.008	0.106 ± 0.015	0.125 ± 0.008	0.102 ± 0.009
Week 14	0.098 ± 0.013	0.165 ± 0.060	0.124 ± 0.008	0.102 ± 0.014	0.104 ± 0.014	0.086 ± 0.011
Eosinophils (10^3/µL)						
Day 4	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.01 ± 0.00
Day 22	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.01	0.01 ± 0.00
Week 14	0.02 ± 0.00	0.08 ± 0.04	0.04 ± 0.01	0.06 ± 0.03	0.02 ± 0.00	0.01 ± 0.00
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	14.9 ± 0.6	15.7 ± 0.7	14.3 ± 0.4	15.4 ± 0.6	17.0 ± 0.7*	19.6 ± 0.6**
Day 22	16.1 ± 0.5	14.6 ± 0.4	14.1 ± 0.5	14.2 ± 0.5	16.0 ± 0.7	15.8 ± 0.6
Week 14	18.5 ± 0.5	16.7 ± 0.5	17.5 ± 0.5	17.2 ± 0.4	16.6 ± 0.4	18.4 ± 0.5
Creatinine (mg/dL)						
Day 4	0.29 ± 0.01	0.28 ± 0.01	0.28 ± 0.01	0.29 ± 0.01	0.27 ± 0.02	0.28 ± 0.01
Day 22	0.33 ± 0.02	0.31 ± 0.01	0.34 ± 0.02	0.34 ± 0.02	0.31 ± 0.01	0.28 ± 0.01
Week 14	0.52 ± 0.02	0.53 ± 0.02	0.53 ± 0.02	0.49 ± 0.02	0.50 ± 0.01	0.49 ± 0.01
Total protein (g/dL)						
Day 4	5.7 ± 0.1	5.7 ± 0.0	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
Day 22	6.4 ± 0.0	6.4 ± 0.1	6.5 ± 0.1	6.5 ± 0.1	6.8 ± 0.1*	6.6 ± 0.1
Week 14	7.3 ± 0.1	7.3 ± 0.1	7.5 ± 0.1	7.8 ± 0.1**	8.0 ± 0.1**	8.3 ± 0.1**

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Albumin (g/dL)						
Day 4	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.3 ± 0.1*
Day 22	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.0**	4.7 ± 0.1**	4.7 ± 0.1**
Week 14	4.9 ± 0.0	4.9 ± 0.0	5.0 ± 0.0*	5.2 ± 0.1**	5.4 ± 0.1**	5.7 ± 0.0**
Alanine aminotransferase (IU/L)						
Day 4	51 ± 2	50 ± 1	53 ± 1	50 ± 2	55 ± 2	46 ± 2
Day 22	44 ± 1	41 ± 1	41 ± 1	39 ± 1**	38 ± 1**	44 ± 1
Week 14	76 ± 6	72 ± 5	70 ± 4	56 ± 3**	40 ± 2**	42 ± 2**
Alkaline phosphatase (IU/L)						
Day 4	626 ± 13	622 ± 8	626 ± 12	622 ± 9	657 ± 10	701 ± 15**
Day 22	476 ± 8	486 ± 11	476 ± 9	441 ± 10*	392 ± 8**	392 ± 10**
Week 14	251 ± 9	234 ± 5	186 ± 16**	218 ± 4**	198 ± 4**	195 ± 3**
Creatine kinase (IU/L)						
Day 4	305 ± 39	270 ± 28	268 ± 20	553 ± 211	440 ± 51	386 ± 33
Day 22	324 ± 74	214 ± 35	235 ± 31	209 ± 31	190 ± 27	247 ± 33
Week 14	219 ± 36	196 ± 18[b]	177 ± 18	262 ± 52	199 ± 31	174 ± 16
Sorbitol dehydrogenase (IU/L)						
Day 4	13 ± 1	11 ± 1	11 ± 1	10 ± 2	13 ± 1	16 ± 1
Day 22	15 ± 1	13 ± 1	17 ± 1	14 ± 1	16 ± 1	23 ± 1**
Week 14	33 ± 2	32 ± 2	30 ± 1	32 ± 2	30 ± 2	33 ± 2
Bile acids (µmol/L)						
Day 4	23.8 ± 1.4	26.5 ± 2.1	26.4 ± 1.3	33.8 ± 2.2**	49.8 ± 6.4**	41.0 ± 5.4**
Day 22	25.7 ± 1.1	25.5 ± 1.2	31.0 ± 2.6	28.0 ± 2.0	34.9 ± 2.9**	39.1 ± 2.8**
Week 14	31.1 ± 3.4	26.0 ± 1.0	29.0 ± 1.9	26.1 ± 0.8	33.1 ± 3.1	39.2 ± 2.9
Female						
Hematology						
n						
Day 4	10	9	10	10	10	9
Day 22	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (%)						
Day 4	42.6 ± 0.5	41.4 ± 0.5	42.6 ± 0.4	40.6 ± 1.1	39.8 ± 0.8*	44.9 ± 0.8
Day 22	44.9 ± 0.3	45.9 ± 0.4	44.4 ± 0.4	43.5 ± 0.3*	42.4 ± 0.6**	42.4 ± 1.1**
Week 14	44.3 ± 0.8	45.2 ± 0.3	45.1 ± 0.3	43.7 ± 0.7	42.6 ± 0.2**	40.7 ± 0.4**
Hemoglobin (g/dL)						
Day 4	14.4 ± 0.2	14.1 ± 0.2	14.4 ± 0.2	13.8 ± 0.4	13.6 ± 0.3	15.3 ± 0.3
Day 22	15.5 ± 0.1	15.9 ± 0.1	15.4 ± 0.1	15.2 ± 0.1	14.8 ± 0.2*	14.7 ± 0.4**
Week 14	15.1 ± 0.2	15.4 ± 0.1	15.4 ± 0.1	15.0 ± 0.2	14.7 ± 0.1*	13.9 ± 0.2**

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Erythrocytes (10^6/µL)						
Day 4	7.33 ± 0.09	7.16 ± 0.09	7.35 ± 0.07	7.02 ± 0.19	6.93 ± 0.15	7.87 ± 0.13
Day 22	7.80 ± 0.07	7.95 ± 0.08	7.75 ± 0.08	7.70 ± 0.06	7.51 ± 0.13	7.74 ± 0.17
Week 14	8.30 ± 0.13	8.50 ± 0.05	8.48 ± 0.07	8.27 ± 0.12	8.17 ± 0.03	7.90 ± 0.07**
Reticulocytes (10^6/µL)						
Day 4	0.36 ± 0.01	0.35 ± 0.03	0.35 ± 0.01	0.36 ± 0.02	0.32 ± 0.02	0.28 ± 0.01**
Day 22	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.01	0.22 ± 0.03	0.29 ± 0.02*
Week 14	0.23 ± 0.02	0.26 ± 0.02	0.21 ± 0.02	0.23 ± 0.02	0.26 ± 0.03	0.33 ± 0.02**
Mean cell volume (fL)						
Day 4	58.0 ± 0.2	57.8 ± 0.2	57.8 ± 0.3	57.8 ± 0.3	57.6 ± 0.2	57.1 ± 0.2**
Day 22	57.5 ± 0.2	57.7 ± 0.2	57.4 ± 0.3	56.6 ± 0.2*	56.6 ± 0.4*	54.6 ± 0.2**
Week 14	53.5 ± 0.2	53.2 ± 0.1	53.2 ± 0.1	52.8 ± 0.1**	52.1 ± 0.2**	51.5 ± 0.2**
Mean cell hemoglobin (pg)						
Day 4	19.7 ± 0.1	19.7 ± 0.1	19.6 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.4 ± 0.1
Day 22	19.9 ± 0.1	20.0 ± 0.1	19.9 ± 0.1	19.8 ± 0.1	19.7 ± 0.1	19.0 ± 0.1**
Week 14	18.2 ± 0.1	18.1 ± 0.1	18.1 ± 0.1	18.1 ± 0.1	18.0 ± 0.1	17.6 ± 0.1**
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.8 ± 0.1	34.0 ± 0.1	33.8 ± 0.1	34.1 ± 0.1	34.3 ± 0.1*	34.0 ± 0.1
Day 22	34.5 ± 0.1	34.6 ± 0.1	34.7 ± 0.1	35.0 ± 0.1*	34.8 ± 0.1	34.7 ± 0.1
Week 14	34.1 ± 0.2	34.1 ± 0.1	34.1 ± 0.1	34.4 ± 0.1	34.5 ± 0.2	34.0 ± 0.1
Platelets (10^3/µL)						
Day 4	491.3 ± 17.4	493.4 ± 26.1	474.1 ± 18.2	530.4 ± 19.1	477.7 ± 22.1	520.8 ± 31.1
Day 22	457.9 ± 30.4	519.9 ± 15.7	494.0 ± 22.7	493.1 ± 13.2	466.1 ± 20.9	450.5 ± 25.3
Week 14	464.9 ± 19.1[b]	460.9 ± 10.7	519.1 ± 23.4	467.5 ± 25.0	481.8 ± 27.0	513.1 ± 21.5
Leukocytes (10^3/µL)						
Day 4	10.23 ± 0.27	9.24 ± 0.57	9.40 ± 0.62	9.09 ± 0.79	9.68 ± 0.68	12.54 ± 0.43*
Day 22	12.19 ± 0.36	12.38 ± 0.32	11.45 ± 0.38	11.81 ± 0.41	11.51 ± 0.44	11.40 ± 0.55
Week 14	9.17 ± 0.53	9.29 ± 0.29	9.15 ± 0.52	9.65 ± 0.47	9.08 ± 0.69	9.74 ± 0.43
Segmented neutrophils (10^3/µL)						
Day 4	0.61 ± 0.03	0.55 ± 0.05	0.60 ± 0.06	0.49 ± 0.05	0.52 ± 0.05	0.68 ± 0.05
Day 22	0.66 ± 0.03	0.63 ± 0.03	0.69 ± 0.06	0.56 ± 0.04	0.51 ± 0.03**	0.52 ± 0.06*
Week 14	0.54 ± 0.03	0.67 ± 0.06	0.74 ± 0.09	0.52 ± 0.03	0.61 ± 0.08	0.58 ± 0.07
Lymphocytes (10^3/µL)						
Day 4	9.37 ± 0.24	8.49 ± 0.50	8.52 ± 0.55	8.36 ± 0.72	8.91 ± 0.62	11.49 ± 0.38
Day 22	11.23 ± 0.32	11.47 ± 0.28	10.45 ± 0.31	11.03 ± 0.36	10.75 ± 0.40	10.66 ± 0.49
Week 14	8.42 ± 0.51	8.40 ± 0.28	8.12 ± 0.42	8.89 ± 0.45	8.23 ± 0.63	8.96 ± 0.39
Monocytes (10^3/µL)						
Day 4	0.12 ± 0.01	0.11 ± 0.02	0.14 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.17 ± 0.02
Day 22	0.13 ± 0.02	0.13 ± 0.01	0.13 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Week 14	0.13 ± 0.02	0.14 ± 0.02	0.17 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	0.14 ± 0.01
Basophils (10^3/µL)						
Day 4	0.113 ± 0.007	0.096 ± 0.010	0.117 ± 0.019	0.102 ± 0.011	0.124 ± 0.015	0.184 ± 0.015**
Day 22	0.147 ± 0.013	0.138 ± 0.009	0.164 ± 0.028	0.116 ± 0.013	0.127 ± 0.016	0.117 ± 0.011
Week 14	0.073 ± 0.018	0.065 ± 0.007	0.085 ± 0.023	0.067 ± 0.006	0.061 ± 0.012	0.058 ± 0.005
Eosinophils (10^3/µL)						
Day 4	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Day 22	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Week 14	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	14.1 ± 0.3	13.7 ± 0.8	13.5 ± 0.9	14.3 ± 0.6	15.4 ± 0.8	16.7 ± 0.6**
Day 22	18.9 ± 0.6	18.9 ± 0.8	18.3 ± 0.7	18.9 ± 0.6	18.8 ± 0.9	19.4 ± 0.7
Week 14	18.3 ± 0.8	18.9 ± 0.5	20.0 ± 0.7	18.0 ± 0.7	18.5 ± 0.5	17.5 ± 0.6
Creatinine (mg/dL)						
Day 4	0.30 ± 0.00	0.29 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.30 ± 0.00	0.27 ± 0.02
Day 22	0.38 ± 0.01	0.43 ± 0.02	0.37 ± 0.02	0.38 ± 0.01	0.38 ± 0.01	0.32 ± 0.02
Week 14	0.51 ± 0.02	0.51 ± 0.01	0.58 ± 0.01*	0.52 ± 0.01	0.49 ± 0.02	0.48 ± 0.01
Total protein (g/dL)						
Day 4	5.9 ± 0.1	5.8 ± 0.0	6.0 ± 0.1	5.9 ± 0.0	5.9 ± 0.1	5.8 ± 0.1
Day 22	6.3 ± 0.1	6.6 ± 0.1	6.5 ± 0.2	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
Week 14	6.9 ± 0.1	7.2 ± 0.1	7.5 ± 0.1**	7.4 ± 0.1**	7.5 ± 0.1**	7.8 ± 0.1**
Albumin (g/dL)						
Day 4	4.3 ± 0.0	4.3 ± 0.0	4.5 ± 0.0*	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.1
Day 22	4.6 ± 0.1	4.7 ± 0.0	4.7 ± 0.1	4.8 ± 0.1**	4.8 ± 0.1*	4.9 ± 0.1**
Week 14	5.1 ± 0.2	5.1 ± 0.1	5.3 ± 0.1*	5.4 ± 0.1**	5.4 ± 0.1**	5.7 ± 0.1**
Alanine aminotransferase (IU/L)						
Day 4	46 ± 1	45 ± 1	48 ± 2	47 ± 1	50 ± 1	44 ± 2
Day 22	35 ± 1	38 ± 1	37 ± 2	36 ± 1	34 ± 1	35 ± 1
Week 14	72 ± 6	75 ± 9	85 ± 8	45 ± 2**	43 ± 2**	31 ± 1**
Alkaline phosphatase (IU/L)						
Day 4	541 ± 8	544 ± 5	557 ± 9	554 ± 9	567 ± 19	575 ± 21
Day 22	416 ± 9	421 ± 9	399 ± 8	392 ± 7	337 ± 13**	356 ± 13**
Week 14	207 ± 10	188 ± 5	177 ± 3**	165 ± 4**	170 ± 10**	146 ± 3**
Creatine kinase (IU/L)						
Day 4	270 ± 44	255 ± 32	298 ± 47[b]	321 ± 55	272 ± 27	374 ± 35
Day 22	275 ± 39	292 ± 59	182 ± 26	227 ± 58	366 ± 77	302 ± 69
Week 14	510 ± 156	388 ± 37	362 ± 57	328 ± 74	526 ± 159	281 ± 42

	0 ppm	**10 ppm**	**30 ppm**	**100 ppm**	**300 ppm**	**1,000 ppm**
Sorbitol dehydrogenase (IU/L)						
Day 4	14 ± 1	15 ± 1	16 ± 1	15 ± 1	16 ± 0	16 ± 1
Day 22	19 ± 2	17 ± 3	20 ± 2	20 ± 3	19 ± 3	18 ± 2
Week 14	35 ± 3	38 ± 1	41 ± 1	35 ± 3	34 ± 2	35 ± 2
Bile acids (µmol/L)						
Day 4	26.4 ± 1.3	24.6 ± 0.8	27.3 ± 2.2	26.9 ± 1.6	40.3 ± 3.8**	31.7 ± 2.8*
Day 22	24.0 ± 0.8	30.1 ± 1.5*	27.0 ± 1.4	23.1 ± 1.1	29.1 ± 1.7	27.2 ± 1.0
Week 14	27.0 ± 1.7	34.3 ± 2.9	32.6 ± 1.4	34.8 ± 3.3	30.8 ± 1.5	36.4 ± 3.2*

*Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test.
**$P \le 0.01$.
[a]Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.
[b]n = 9.

Table B-2. Hematology Data for Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Hematocrit (%)	51.2 ± 0.4	51.4 ± 0.5	50.4 ± 0.7	50.2 ± 0.4	50.3 ± 0.6	50.5 ± 0.6
Hemoglobin (g/dL)	16.4 ± 0.1	16.5 ± 0.1	16.1 ± 0.2	16.1 ± 0.1	16.2 ± 0.2	16.2 ± 0.2
Erythrocytes (10^6/µL)	10.63 ± 0.09	10.78 ± 0.10	10.46 ± 0.13	10.65 ± 0.07	10.67 ± 0.11	10.69 ± 0.08
Reticulocytes (10^6/µL)	0.25 ± 0.02	0.27 ± 0.02	0.29 ± 0.03	0.28 ± 0.02	0.28 ± 0.03	0.26 ± 0.03
Mean cell volume (fL)	47.3 ± 0.3	47.3 ± 0.3	47.5 ± 0.2	46.8 ± 0.1	47.0 ± 0.4	46.7 ± 0.2
Mean cell hemoglobin (pg)	15.2 ± 0.1	15.2 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.2	32.1 ± 0.1	32.0 ± 0.1	32.2 ± 0.1	32.2 ± 0.1	32.1 ± 0.1
Platelets (10^3/µL)	585.6 ± 24.8	569.5 ± 15.9	580.9 ± 17.8	572.3 ± 17.3	549.7 ± 21.3	549.8 ± 17.9
Leukocytes (10^3/µL)	6.13 ± 0.35	6.39 ± 0.56	5.91 ± 0.34	6.07 ± 0.31	5.88 ± 0.28	5.95 ± 0.39
Segmented neutrophils (10^3/µL)	0.25 ± 0.02	0.28 ± 0.05	0.25 ± 0.03	0.28 ± 0.03	0.26 ± 0.03	0.27 ± 0.04
Lymphocytes (10^3/µL)	5.68 ± 0.33	5.86 ± 0.49	5.48 ± 0.30	5.56 ± 0.29	5.39 ± 0.29	5.44 ± 0.34
Monocytes (10^3/µL)	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.17 ± 0.05	0.14 ± 0.02
Basophils (10^3/µL)	0.031 ± 0.006	0.065 ± 0.025	0.033 ± 0.005	0.041 ± 0.005	0.034 ± 0.004	0.067 ± 0.023
Eosinophils (10^3/µL)	0.02 ± 0.00	0.04 ± 0.02	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.04 ± 0.01
Female						
n	9	10	10	10	10	10
Hematocrit (%)	50.9 ± 1.1	50.5 ± 0.7	48.9 ± 0.4	50.5 ± 0.4	48.8 ± 0.6	46.6 ± 0.4**
Hemoglobin (g/dL)	16.2 ± 0.3	16.0 ± 0.2	15.7 ± 0.1	16.2 ± 0.1	15.7 ± 0.2	15.1 ± 0.1**
Erythrocytes (10^6/µL)	10.56 ± 0.20	10.42 ± 0.12	10.23 ± 0.09	10.54 ± 0.07	10.22 ± 0.13	9.96 ± 0.09**
Reticulocytes (10^6/µL)	0.40 ± 0.03	0.33 ± 0.03	0.32 ± 0.03	0.37 ± 0.03	0.37 ± 0.03	0.31 ± 0.03
Mean cell volume (fL)	48.2 ± 0.2	48.2 ± 0.1	47.9 ± 0.2	47.9 ± 0.2	47.7 ± 0.2	46.9 ± 0.2**
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.3 ± 0.0	15.3 ± 0.1	15.4 ± 0.1	15.4 ± 0.0	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.8 ± 0.1	31.7 ± 0.1	32.0 ± 0.1	32.0 ± 0.1	32.2 ± 0.1	32.4 ± 0.1**
Platelets (10^3/µL)	461.3 ± 32.5	485.2 ± 28.8	518.3 ± 26.1	491.6 ± 24.8	552.1 ± 20.5*	569.4 ± 23.2*
Leukocytes (10^3/µL)	4.18 ± 0.41	4.10 ± 0.24	4.07 ± 0.27	4.22 ± 0.31	4.16 ± 0.19	4.56 ± 0.27
Segmented neutrophils (10^3/µL)	0.21 ± 0.04	0.14 ± 0.02	0.21 ± 0.07	0.16 ± 0.02	0.13 ± 0.01	0.15 ± 0.02
Lymphocytes (10^3/µL)	3.80 ± 0.34	3.87 ± 0.23	3.73 ± 0.22	3.96 ± 0.29	3.93 ± 0.19	4.31 ± 0.27
Monocytes (10^3/µL)	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Basophils (10^3/µL)	0.050 ± 0.017	0.019 ± 0.006	0.020 ± 0.007	0.024 ± 0.008	0.019 ± 0.007	0.011 ± 0.002
Eosinophils (10^3/µL)	0.03 ± 0.01	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.00

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

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

Tables

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Table C-1. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Two-week Dermal Study of *o*-Chloropyridine[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	157 ± 6	159 ± 6	157 ± 4	153 ± 3	158 ± 5	158 ± 4
Heart						
Absolute	0.60 ± 0.02	0.61 ± 0.02	0.59 ± 0.02	0.60 ± 0.01	0.65 ± 0.04	0.60 ± 0.01
Relative	3.819 ± 0.079	3.829 ± 0.074	3.752 ± 0.080	3.920 ± 0.045	4.131 ± 0.250	3.823 ± 0.100
R. Kidney						
Absolute	0.69 ± 0.03	0.71 ± 0.03	0.72 ± 0.02	0.68 ± 0.03	0.73 ± 0.03	0.73 ± 0.02
Relative	4.408 ± 0.089	4.439 ± 0.073	4.572 ± 0.059	4.436 ± 0.152	4.623 ± 0.084	4.604 ± 0.104
Liver						
Absolute	8.02 ± 0.28	8.13 ± 0.18	8.25 ± 0.23	8.03 ± 0.16	8.87 ± 0.35*	8.79 ± 0.11*
Relative	51.083 ± 0.188	51.251 ± 0.857	52.473 ± 1.018	52.428 ± 0.422	55.961 ± 0.567**	55.732 ± 0.777**
Lung						
Absolute	1.00 ± 0.05	1.13 ± 0.16	1.06 ± 0.06	0.98 ± 0.04	1.09 ± 0.07	0.79 ± 0.18
Relative	6.353 ± 0.107	7.001 ± 0.731	6.725 ± 0.277	6.413 ± 0.420	6.866 ± 0.421	5.088 ± 1.133
R. Testis						
Absolute	0.996 ± 0.035	1.001 ± 0.034	1.028 ± 0.022	0.970 ± 0.036	1.011 ± 0.031	0.983 ± 0.047
Relative	6.346 ± 0.134	6.296 ± 0.096	6.559 ± 0.269	6.324 ± 0.128	6.391 ± 0.136	6.217 ± 0.180
Thymus						
Absolute	0.471 ± 0.022	0.463 ± 0.018	0.455 ± 0.010	0.483 ± 0.015	0.475 ± 0.016	0.466 ± 0.021
Relative	2.999 ± 0.099	2.912 ± 0.068	2.898 ± 0.054	3.151 ± 0.038	3.012 ± 0.127	2.958 ± 0.148
Female						
Necropsy body wt	120 ± 2	121 ± 1	120 ± 3	122 ± 4	120 ± 2	120 ± 2
Heart						
Absolute	0.48 ± 0.01	0.50 ± 0.02	0.51 ± 0.01	0.49 ± 0.02	0.48 ± 0.01	0.50 ± 0.01
Relative	4.007 ± 0.098	4.094 ± 0.135	4.205 ± 0.060	4.041 ± 0.054	4.009 ± 0.112	4.120 ± 0.059
R. Kidney						
Absolute	0.57 ± 0.02	0.61 ± 0.01	0.59 ± 0.01	0.60 ± 0.02	0.58 ± 0.01	0.58 ± 0.01
Relative	4.729 ± 0.050	4.994 ± 0.095	4.929 ± 0.066	4.945 ± 0.051	4.844 ± 0.060	4.798 ± 0.109
Liver						
Absolute	5.64 ± 0.14	5.85 ± 0.17	5.91 ± 0.17	6.03 ± 0.30	5.69 ± 0.10	6.20 ± 0.11b
Relative	47.053 ± 1.335	48.238 ± 1.369	49.157 ± 1.602	49.495 ± 0.914	47.564 ± 0.707	51.537 ± 0.476[b]
Lung						
Absolute	0.81 ± 0.02	0.84 ± 0.02	0.85 ± 0.05	0.82 ± 0.02	0.83 ± 0.02	0.84 ± 0.03
Relative	6.716 ± 0.216	6.955 ± 0.174	7.036 ± 0.365	6.754 ± 0.120	6.901 ± 0.210	6.979 ± 0.222

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
Thymus						
Absolute	0.374 ± 0.012	0.411 ± 0.025	0.385 ± 0.011	0.379 ± 0.027	0.390 ± 0.008	0.371 ± 0.018
Relative	3.115 ± 0.104	3.389 ± 0.212	3.202 ± 0.090	3.105 ± 0.122	3.258 ± 0.076	3.080 ± 0.097

*Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' 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).
[b]n = 4.

Table C-2. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
n	10	10	10	10	10	10
Male						
Necropsy body wt	320 ± 6	326 ± 7	326 ± 8	326 ± 8	309 ± 7	268 ± 6**
Heart						
Absolute	0.90 ± 0.02	0.93 ± 0.03	0.90 ± 0.03[b]	0.94 ± 0.03	0.90 ± 0.02	0.83 ± 0.01
Relative	2.806 ± 0.032	2.854 ± 0.048	2.791 ± 0.022[b]	2.890 ± 0.079	2.898 ± 0.032	3.089 ± 0.046**
R. Kidney						
Absolute	0.95 ± 0.01	1.02 ± 0.02*	1.04 ± 0.03*	1.01 ± 0.02*	1.02 ± 0.03*	1.07 ± 0.02**
Relative	2.978 ± 0.065	3.144 ± 0.036*	3.172 ± 0.052*	3.110 ± 0.081	3.304 ± 0.030**	4.012 ± 0.052**
Liver						
Absolute	10.91 ± 0.29	11.76 ± 0.29	10.87 ± 0.78	13.17 ± 0.35**	13.51 ± 0.40**	14.33 ± 0.36**
Relative	34.101 ± 0.737	36.114 ± 0.615	33.092 ± 1.788	40.530 ± 1.053**	43.672 ± 0.620**	53.553 ± 0.653**
Lung						
Absolute	1.34 ± 0.05	1.35 ± 0.04	1.45 ± 0.09	1.32 ± 0.05	1.32 ± 0.04	1.21 ± 0.05
Relative	4.186 ± 0.150	4.124 ± 0.069	4.422 ± 0.185	4.061 ± 0.154	4.261 ± 0.113	4.493 ± 0.114
R. Testis						
Absolute	1.344 ± 0.030	1.394 ± 0.029	1.404 ± 0.021	1.457 ± 0.028*	1.421 ± 0.025	1.423 ± 0.022
Relative	4.212 ± 0.118	4.282 ± 0.051	4.323 ± 0.100	4.490 ± 0.121	4.611 ± 0.090**	5.330 ± 0.091**
Thymus						
Absolute	0.317 ± 0.014	0.327 ± 0.009	0.317 ± 0.010	0.314 ± 0.011	0.309 ± 0.014	0.256 ± 0.008**
Relative	0.990 ± 0.040	1.005 ± 0.032	0.979 ± 0.043	0.968 ± 0.039	0.999 ± 0.038	0.959 ± 0.030
Female						
Necropsy body wt	195 ± 4	200 ± 2	200 ± 4	196 ± 2	190 ± 3	174 ± 2**
Heart						
Absolute	0.57 ± 0.01	0.58 ± 0.01	0.61 ± 0.02	0.60 ± 0.02	0.59 ± 0.01	0.57 ± 0.01
Relative	2.915 ± 0.034	2.907 ± 0.035	3.043 ± 0.065	3.074 ± 0.083	3.093 ± 0.064*	3.249 ± 0.031**
R. Kidney						
Absolute	0.65 ± 0.02	0.67 ± 0.01	0.70 ± 0.01*	0.69 ± 0.02*	0.71 ± 0.01**	0.74 ± 0.01**
Relative	3.317 ± 0.047	3.347 ± 0.032	3.472 ± 0.050*	3.530 ± 0.051**	3.714 ± 0.058**	4.259 ± 0.040**
Liver						
Absolute	5.80 ± 0.22	6.14 ± 0.14	6.77 ± 0.17**	6.89 ± 0.12**	7.94 ± 0.34**	9.06 ± 0.21**
Relative	29.632 ± 0.735	30.698 ± 0.619	33.784 ± 0.410**	35.152 ± 0.373**	41.684 ± 1.375**	52.086 ± 0.597**
Lung						
Absolute	0.93 ± 0.02	0.95 ± 0.02	0.96 ± 0.02	0.96 ± 0.02	0.95 ± 0.02	0.87 ± 0.01
Relative	4.760 ± 0.095	4.745 ± 0.118	4.818 ± 0.118	4.914 ± 0.074	5.002 ± 0.103	5.027 ± 0.091
Thymus						
Absolute	0.235 ± 0.008	0.242 ± 0.009	0.240 ± 0.006	0.247 ± 0.016	0.243 ± 0.010	0.206 ± 0.006
Relative	1.206 ± 0.037	1.211 ± 0.044	1.198 ± 0.031	1.265 ± 0.087	1.276 ± 0.046	1.189 ± 0.037

*Significantly different ($P \leq 0.05$) from the control group by Williams' 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).
[b]n = 9.

Table C-3. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Two-week Dermal Study of *o*-Chloropyridine[a]

	Vehicle Control	6.25 mg/kg	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.7 ± 0.3	26.4 ± 0.5	26.8 ± 0.4	26.1 ± 0.5	25.7 ± 0.3	25.6 ± 0.7
Heart						
Absolute	0.13 ± 0.01	0.13 ± 0.00	0.14 ± 0.00	0.14 ± 0.01	0.13 ± 0.00	0.13 ± 0.01
Relative	5.009 ± 0.181	5.046 ± 0.070	5.088 ± 0.079	5.218 ± 0.136	5.230 ± 0.069	5.182 ± 0.129
R. Kidney						
Absolute	0.26 ± 0.00	0.27 ± 0.01	0.28 ± 0.00	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.01
Relative	10.059 ± 0.254	10.379 ± 0.134	10.608 ± 0.230	10.544 ± 0.316	10.940 ± 0.277*	10.921 ± 0.248*
Liver						
Absolute	1.48 ± 0.02	1.51 ± 0.02	1.51 ± 0.03	1.50 ± 0.04	1.50 ± 0.01	1.53 ± 0.04
Relative	57.443 ± 0.881	57.168 ± 0.753	56.504 ± 1.101	57.429 ± 1.473	58.181 ± 0.698	59.948 ± 1.673
Lung						
Absolute	0.16 ± 0.01	0.20 ± 0.02	0.18 ± 0.02	0.18 ± 0.01	0.17 ± 0.01	0.18 ± 0.01
Relative	6.095 ± 0.472	7.599 ± 0.625	6.870 ± 0.831	6.827 ± 0.514	6.716 ± 0.303	6.926 ± 0.305
R. Testis						
Absolute	0.110 ± 0.003	0.105 ± 0.004	0.106 ± 0.004	0.108 ± 0.003	0.113 ± 0.003	0.103 ± 0.004
Relative	4.265 ± 0.131	3.974 ± 0.153	3.979 ± 0.158	4.130 ± 0.068	4.390 ± 0.118	4.042 ± 0.084
Thymus						
Absolute	0.054 ± 0.003	0.054 ± 0.001	0.052 ± 0.003	0.046 ± 0.001	0.053 ± 0.002	0.054 ± 0.005
Relative	2.101 ± 0.104	2.036 ± 0.040	1.940 ± 0.090	1.774 ± 0.067	2.066 ± 0.096	2.103 ± 0.150
Female						
Necropsy body wt	23.2 ± 0.6	23.1 ± 0.4	22.8 ± 0.6	23.0 ± 0.6	22.7 ± 0.6	23.3 ± 0.4
Heart						
Absolute	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
Relative	5.411 ± 0.164	5.498 ± 0.130	5.589 ± 0.091	5.679 ± 0.052	5.646 ± 0.140	5.595 ± 0.099
R. Kidney						
Absolute	0.20 ± 0.01	0.20 ± 0.01	0.20 ± 0.00	0.21 ± 0.01	0.20 ± 0.01	0.21 ± 0.01
Relative	8.383 ± 0.185	8.835 ± 0.119	8.598 ± 0.185	9.074 ± 0.133*	8.613 ± 0.194	8.849 ± 0.172
Liver						
Absolute	1.37 ± 0.05	1.36 ± 0.06	1.28 ± 0.04	1.38 ± 0.08	1.32 ± 0.04	1.47 ± 0.01
Relative	58.806 ± 0.979	58.610 ± 1.496	56.121 ± 1.307	60.054 ± 2.197	58.148 ± 0.773	63.336 ± 1.070
Lung						
Absolute	0.18 ± 0.01	0.21 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Relative	7.763 ± 0.474	8.875 ± 0.383	8.060 ± 0.386	8.176 ± 0.604	7.969 ± 0.324	7.576 ± 0.297
Thymus						
Absolute	0.075 ± 0.003	0.070 ± 0.002	0.066 ± 0.002	0.071 ± 0.003	0.079 ± 0.004	0.077 ± 0.006
Relative	3.231 ± 0.083	3.056 ± 0.136	2.900 ± 0.079	3.107 ± 0.202	3.475 ± 0.089	3.316 ± 0.194

*Significantly different ($P \leq 0.05$) from the vehicle 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 C-4. Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Male						
n	10	10	10	10	10	10
Necropsy body wt	40.8 ± 0.9	42.4 ± 1.1	42.4 ± 0.6	40.2 ± 1.2	40.5 ± 1.2	33.0 ± 0.5**
Heart						
Absolute	0.17 ± 0.00	0.17 ± 0.00	0.18 ± 0.00	0.17 ± 0.00	0.17 ± 0.00	0.15 ± 0.00**
Relative	4.116 ± 0.070	4.108 ± 0.112	4.293 ± 0.089	4.169 ± 0.091	4.129 ± 0.105	4.573 ± 0.059**
R. Kidney						
Absolute	0.34 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.31 ± 0.01
Relative	8.237 ± 0.218	8.203 ± 0.090	8.193 ± 0.244	8.631 ± 0.217	8.556 ± 0.229	9.242 ± 0.369*
Liver						
Absolute	1.70 ± 0.06	1.89 ± 0.07*	1.95 ± 0.04*	1.84 ± 0.07*	2.01 ± 0.08**	1.95 ± 0.05**
Relative	41.624 ± 0.778	44.531 ± 0.718*	45.851 ± 0.819**	45.766 ± 0.974**	49.467 ± 0.796**	59.121 ± 0.830**
Lung						
Absolute	0.28 ± 0.02	0.27 ± 0.02	0.26 ± 0.02	0.27 ± 0.02	0.29 ± 0.02	0.29 ± 0.01
Relative	6.824 ± 0.375	6.479 ± 0.445	6.230 ± 0.489	6.806 ± 0.423	7.248 ± 0.507	8.676 ± 0.441**
R. Testis						
Absolute	0.119 ± 0.001	0.119 ± 0.002	0.121 ± 0.002	0.117 ± 0.002	0.120 ± 0.003	0.116 ± 0.004
Relative	2.926 ± 0.047	2.810 ± 0.067	2.857 ± 0.046	2.945 ± 0.102	2.969 ± 0.079	3.502 ± 0.093**
Thymus						
Absolute	0.054 ± 0.004	0.061 ± 0.006[b]	0.054 ± 0.002	0.052 ± 0.002	0.057 ± 0.004	0.043 ± 0.002
Relative	1.328 ± 0.075	1.428 ± 0.123[b]	1.264 ± 0.054	1.293 ± 0.047	1.401 ± 0.097	1.308 ± 0.069
Female						
n	9	10	10	10	10	10
Necropsy body wt	28.5 ± 1.4	31.8 ± 1.0	31.2 ± 1.3	30.3 ± 1.1	31.9 ± 0.8	28.4 ± 0.6
Heart						
Absolute	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.00	0.12 ± 0.00	0.13 ± 0.00	0.13 ± 0.00
Relative	4.511 ± 0.153	4.038 ± 0.143*	4.181 ± 0.134	4.130 ± 0.131	4.102 ± 0.092	4.494 ± 0.063
R. Kidney						
Absolute	0.19 ± 0.01	0.20 ± 0.00	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.00	0.22 ± 0.01**
Relative	6.866 ± 0.352	6.203 ± 0.206	6.212 ± 0.170	6.232 ± 0.174	6.321 ± 0.154	7.676 ± 0.156*
Liver						
Absolute	1.22 ± 0.09	1.37 ± 0.04	1.34 ± 0.05	1.35 ± 0.07	1.62 ± 0.05**	1.94 ± 0.0.05**
Relative	42.778 ± 1.560	43.065 ± 1.066	43.266 ± 0.645	44.466 ± 1.122	50.818 ± 1.290**	68.154 ± 1.026**

	0 ppm	10 ppm	30 ppm	100 ppm	300 ppm	1,000 ppm
Lung						
Absolute	0.25 ± 0.02	0.27 ± 0.02	0.25 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.23 ± 0.01
Relative	8.952 ± 0.699	8.502 ± 0.795	7.981 ± 0.537	7.523 ± 0.440	7.535 ± 0.324	7.987 ± 0.380
Thymus						
Absolute	0.049 ± 0.004	0.056 ± 0.003	0.054 ± 0.003	0.054 ± 0.002	0.053 ± 0.002	0.045 ± 0.002
Relative	1.715 ± 0.111	1.762 ± 0.123	1.722 ± 0.077	1.812 ± 0.073	1.679 ± 0.072	1.578 ± 0.077

*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).
[b]n = 9.

Appendix D. Reproductive Tissue Evaluations and Estrous Cycle Characterization

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Table D-1. Summary of Reproductive Tissue Evaluations for Male Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	100 ppm	300 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	320 ± 6	326 ± 8	309 ± 7	268 ± 6**
L. Cauda epididymis	0.1510 ± 0.0067	0.1563 ± 0.0040	0.1548 ± 0.0041	0.1503 ± 0.0037
L. Epididymis	0.4239 ± 0.0177	0.4579 ± 0.0097	0.4457 ± 0.0063	0.4222 ± 0.0079
L. Testis	1.4547 ± 0.0387	1.5359 ± 0.0227	1.4795 ± 0.0269	1.4671 ± 0.0212
Spermatid measurements				
Spermatid heads (10^6/testis)	169.75 ± 7.20	182.13 ± 5.88	170.88 ± 4.14	166.75 ± 4.25
Spermatid heads (10^6/g testis)	129.7 ± 3.6	131.9 ± 4.6	126.8 ± 2.8	125.6 ± 3.0
Epididymal spermatozoal measurements				
Sperm motility (%)	77.6 ± 1.0	71.7 ± 8.0	76.9 ± 1.4	77.4 ± 1.0
Sperm (10^6/cauda epididymis)	60.00 ± 4.43	68.75 ± 6.84	62.15 ± 5.53	42.40 ± 2.02*
Sperm (10^6/g cauda epididymis)	402 ± 29	443 ± 46	400 ± 32	283 ± 13*

*Significantly different ($P \leq 0.05$) from the control group by Shirley's or Dunn'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 (tissue weights) or Dunn's test (spermatid measurements and sperm motility).

Table D-2. Estrous Cycle Characterization for Female Rats in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	100 ppm	300 ppm	1,000 ppm
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	195 ± 4	196 ± 2	190 ± 3	174 ± 2**
Proportion of regular cycling females[b]	9/10	10/10	8/10	9/10
Estrous cycle length (days)	5.50 ± 0.27	5.00 ± 0.15	5.50 ± 0.24[c]	5.90 ± 0.18
Estrous stages (% of cycle)				
Diestrus	53.3	54.2	62.5	61.7
Proestrus	15.0	14.2	11.7	13.3
Estrus	23.3	20.8	20.8	19.2
Metestrus	8.3	10.8	5.0	5.8

**Significantly different ($P \leq 0.01$) 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 among all groups and between the control group and each exposed group indicated 300 ppm females had a higher proportion of extended diestrus than control females.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.

Table D-3. Results of Vaginal Cytology Study Using the Transition Matrix Approach for Female Rats in the Three-month Drinking Water Study of *o*-Chloropyridine

Stage	Comparison	P Value	Trend[a]
Overall tests	Overall	0.024	
Overall tests	100 ppm vs. controls	0.257	N
Overall tests	300 ppm vs. controls	0.009	–
Overall tests	1,000 vs. controls	0.32	–
Extended estrus	Overall	0.721	
Extended estrus	100 ppm vs. controls	0.305	N
Extended estrus	300 ppm vs. controls	0.595	N
Extended estrus	1,000 vs. controls	0.878	N
Extended diestrus	Overall	0.039	
Extended diestrus	100 ppm vs. controls	0.695	N
Extended diestrus	300 ppm vs. controls	0.008	–
Extended diestrus	1,000 vs. controls	0.249	–
Extended metestrus	Overall	1	
Extended metestrus	100 ppm vs. controls	1	–
Extended metestrus	300 ppm vs. controls	1	–
Extended metestrus	1,000 vs. controls	1	–
Extended proestrus	Overall	1	
Extended proestrus	100 ppm vs. controls	1	–
Extended proestrus	300 ppm vs. controls	1	–
Extended Proestrus	1,000 vs. controls	1	–
Skipped estrus	Overall	1	
Skipped estrus	100 ppm vs. controls	1	–
Skipped estrus	300 ppm vs. controls	1	–
Skipped estrus	1,000 vs. controls	1	–
Skipped diestrus	Overall	1	
Skipped diestrus	100 ppm vs. controls	1	–
Skipped diestrus	300 ppm vs. controls	1	–
Skipped diestrus	1,000 vs. controls	1	–
Overall tests	300 ppm vs. controls	0.009	–
Extended diestrus	300 ppm vs. controls	0.008	–

[a]N means that the exposed group had a lower probability of transitioning to and/or from the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the control group.

Table D-4. Summary of Reproductive Tissue Evaluations for Male Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	100 ppm	300 ppm	1,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body wt	40.8 ± 0.9	40.2 ± 1.2	40.5 ± 1.2	33.0 ± 0.5**
L. Cauda epididymis	0.0200 ± 0.0010	0.0194 ± 0.0012	0.0173 ± 0.0008	0.0201 ± 0.0009
L. Epididymis	0.0509 ± 0.0019	0.0545 ± 0.0017	0.0548 ± 0.0019	0.0530 ± 0.0020
L. Testis	0.1133 ± 0.0020	0.1132 ± 0.0012	0.1158 ± 0.0022	0.1101 ± 0.0022
Spermatid measurements				
Spermatid heads (10^6/testis)	19.87 ± 0.88	19.39 ± 0.91	20.32 ± 0.60	19.84 ± 0.70
Spermatid heads (10^6/g testis)	195.3 ± 9.0	186.4 ± 8.0	190.1 ± 2.2	194.8 ± 5.2
Epididymal spermatozoal measurements				
Sperm motility (%)	74.1 ± 1.3	72.8 ± 3.7	72.0 ± 1.7	69.6 ± 2.7
Sperm (10^6/cauda epididymis)	8.99 ± 1.25	10.29 ± 0.95	6.92 ± 0.80	8.99 ± 1.36
Sperm (10^6/g cauda epididymis)	460 ± 68	554 ± 64	403 ± 43	444 ± 61

**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 (tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

Table D-5. Estrous Cycle Characterization for Female Mice in the Three-month Drinking Water Study of *o*-Chloropyridine[a]

	0 ppm	100 ppm	300 ppm	1,000 ppm
Number weighed at necropsy	9	10	10	10
Necropsy body wt (g)	28.5 ± 1.4	30.3 ± 1.1	31.9 ± 0.8	28.4 ± 0.6
Proportion of regular cycling females[b]	6/9	6/10	8/10	6/10
Estrous cycle length (days)	3.86 ± 0.14[c]	4.45 ± 0.17	3.91 ± 0.14	5.05 ± 0.59
Estrous stages (% of cycle)				
Diestrus	42.6	30.8	32.5	36.7
Proestrus	0.0	0.0	0.0	0.0
Estrus	39.8	47.5	46.7	42.5
Metestrus	17.6	21.7	20.8	20.8

[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 among all groups and between the control group and each exposed group indicated exposed females did not have significantly higher proportions of extended estrus or diestrus than control females.
[b]Number of females with a regular cycle/number of females cycling.
[c]Estrous cycle was longer than 12 days or unclear in 2 of 9 animals.

Table D-6. Results of Vaginal Cytology Study Using the Transition Matrix Approach for Female Mice in the Three-month Drinking Water Study of *o*-Chloropyridine

Stage	Comparison	P Value	Trend[a]
Overall tests	Overall	<0.001	
Overall tests	100 ppm vs. controls	<0.001	–
Overall tests	300 ppm vs. controls	0.088	N
Overall tests	1,000 vs. controls	0.048	–
Extended estrus	Overall	0.236	
Extended estrus	100 ppm vs. controls	0.234	–
Extended estrus	300 ppm vs. controls	0.361	–
Extended estrus	1,000 vs. controls	0.214	–
Extended diestrus	Overall	0.074	
Extended diestrus	100 ppm vs. controls	0.033	N
Extended diestrus	300 ppm vs. controls	0.131	N
Extended diestrus	1,000 vs. controls	0.748	N
Extended metestrus	Overall	1	
Extended metestrus	100 ppm vs. controls	1	–
Extended metestrus	300 ppm vs. controls	1	–
Extended metestrus	1,000 vs. controls	1	–
Extended proestrus	Overall	1	
Extended proestrus	100 ppm vs. controls	1	–
Extended proestrus	300 ppm vs. controls	1	–
Extended Proestrus	1,000 vs. controls	1	–
Skipped estrus	Overall	1	
Skipped estrus	100 ppm vs. controls	1	–
Skipped estrus	300 ppm vs. controls	1	–
Skipped estrus	1,000 vs. controls	1	–
Skipped diestrus	Overall	0.521	
Skipped diestrus	100 ppm vs. controls	0.289	N
Skipped diestrus	300 ppm vs. controls	0.289	N
Skipped diestrus	1,000 vs. controls	0.916	N
Overall tests	100 ppm vs. controls	<0.001	–
Overall tests	1,000 ppm vs. controls	0.048	–
Extended diestrus	100 ppm vs. controls	0.033	N

[a]N means that the exposed group had a lower probability of transitioning to and/or from the relevant abnormal state (extended estrus, extended metestrus, extended proestrus, skipped estrus, or skipped diestrus) than did the control group.

Concentration (PPM)																			
0						E	E	D	D	D	P	E	M	D	D	E	E		
0							E	D	D	D	P	E	D	D	D	E	E	D	
0						P	E	D	D	D	P	E	D	D	D	P	E		
0							E	D	D	D	P	E	M	D	D	P	E	M	
0		D	D	D	P	E	M	D	D	D	D	E	E						
0			M	D	D	D	E	M	D	D	P	E	D	D					
0					D	P	E	D	D	D	P	E	M	D	D	P			
0				D	P	E	D	D	D	D	P	E	M	D	D				
0					D	P	E	D	D	D	P	E	M	D	D	P			
0			D	P	E	D	D	D	D	D	D	E	M	D					
100					M	D	D	E	M	D	D	E	M	D	D	P			
100							E	M	D	D	P	E	M	D	D	E	E	M	
100			M	D	D	P	E	D	D	D	P	E	D	D					
100						P	E	M	D	D	P	E	D	D	D	D	E		
100							E	D	D	D	P	E	D	D	D	P	E	M	
100					D	P	E	D	D	D	P	E	M	D	D	P			
100				D	D	P	E	M	D	D	D	E	D	D	D				
100							E	M	D	D	P	E	D	D	D	P	E	D	
100				D	P	E	D	D	D	D	P	E	D	D	D				
100			D	D	D	D	E	M	D	D	P	E	D	D					
300							E	D	D	D	P	E	D	D	D	P	E	D	
300						P	E	D	D	D	P	E	D	D	D	P	E		
300						E	D	D	D	D	D	E	M	D	D	P	E		
300					D	D	E	E	D	D	D	E	E	D	D	D			
300		D	D	P	E	M	D	D	D	D	P	E	D						
300					D	D	E	D	D	D	D	E	M	D	D	P			
300	E	M	D	D	D	D	D	D	D	D	D	E							
300					P	E	D	D	D	D	P	E	M	D	D	D			
300					P	E	D	D	D	D	P	E	D	D	D	D			
300			D	D	D	D	E	M	D	D	P	E	D	D					
1,000				D	P	E	M	D	D	D	P	E	M	D	D				
1,000			D	D	P	E	D	D	D	D	P	E	D	D					
1,000		D	D	D	D	P	E	M	D	D	D	E	E						
1,000				D	P	E	M	D	D	D	P	E	D	D	D				
1,000					D	P	E	D	D	D	D	E	E	M	D	D			
1,000	M	D	D	P	E	D	D	D	D	D	D	E							
1,000		D	D	D	D	P	E	D	D	D	P	E	D						
1,000		D	D	D	P	E	D	D	D	D	P	E	D						
1,000				D	D	P	E	D	D	D	D	E	D	D	D				
1,000						E	M	D	D	D	P	E	E	D	D	D	P		

Figure D-1. Vaginal Cytology Plots for Female Rats in the Three-month Drinking Water Study of *o*-Chloropyridine

Daily vaginal lavage samples were collected from each animal and estrous stage determined based on vaginal cytology. Individual females are aligned by their second estrus, and color coded based on estrous stage to aid in visual comparisons amongst the groups. D = diestrus, P = proestrus, E = estrus, M = metestrus.

Concentration (PPM)																						
0								E	E	M	D	E	E	M	D	E	E	D	D			
0	D	D	D	D	D	D	D	E	E	M	D	E										
0									E	M	D	E	E	M	D	E	E	M	D	E		
0		E	M	D	D	D	D	D	D	D	D	E	E									
0	E	E	M	D	D	D	D	D	D	D	D	E										
0									E	E	M	E	E	M	D	D	E	E	M	D		
0									E	M	D	E	E	M	D	E	E	M	D	D		
0									E	M	D	E	E	M	D	E	E	M	D	E		
0									E	M	D	E	E	M	D	E	E	D	D	D		
100								E	E	M	D	E	E	M	D	E	E	M	D			
100						M	D	E	E	M	D	E	E	E	D	D	D					
100									E	M	D	E	E	M	D	D	E	E	M	D		
100						M	D	E	E	M	D	E	E	M	D	E	E					
100					M	D	E	E	E	M	D	E	E	E	M	D						
100									E	M	D	E	E	E	M	D	D	E	E	M		
100							D	E	E	M	D	E	E	M	D	E	E	M				
100				D	D	D	E	E	M	D	D	E	E	D	E							
100								E	E	M	D	E	E	M	D	D	E	E	M			
100					E	E	E	E	M	D	D	E	E	M	D	D						
300							D	E	E	M	D	E	E	M	D	D	E	D				
300								E	E	M	D	E	E	M	D	E	E	M	D			
300									E	M	D	E	E	M	D	E	E	D	D	E		
300								E	E	M	D	E	E	M	D	D	E	E	M			
300						M	D	E	E	M	D	E	E	M	D	E	M					
300							D	E	E	M	D	E	M	D	D	D	D	D				
300									E	M	D	E	E	M	D	E	E	D	D	E		
300						M	D	E	E	M	D	E	E	M	D	D	E					
300								E	E	M	D	E	E	M	D	E	E	D	D			
300							E	E	E	M	D	E	E	E	M	D	E	E				
1,000									E	M	D	E	E	M	D	E	E	M	D	D		
1,000								E	E	M	D	E	E	M	D	D	D	E	E			
1,000									E	M	D	E	E	M	D	E	E	M	D	D		
1,000								E	E	M	D	E	M	D	D	E	E	M	D			
1,000		D	E	E	M	D	D	D	D	D	D	E	M									
1,000									E	E	M	E	E	E	M	D	D	D	D	D		
1,000		E	E	M	D	D	D	D	D	D	D	E	M									
1,000							E	E	E	M	D	E	E	E	M	D	E	E				
1,000									E	M	D	E	E	M	D	E	E	M	D	D		
1,000								E	E	M	D	E	E	M	D	E	E	M	D			

Figure D-2. Vaginal Cytology Plots for Female Mice in the Three-month Drinking Water Study of *o*-Chloropyridine

Daily vaginal lavage samples were collected from each animal and estrous stage determined based on vaginal cytology. Individual females are aligned by their second estrus, and color coded based on estrous stage to aid in visual comparisons amongst the groups. D = diestrus, P = proestrus, E = estrus, M = metestrus.

Appendix E. Genetic Toxicology

Tables

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Table E-1. Mutagenicity of *o*-Chloropyridine in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Without S9	With 30% Hamster S9	With 30% Hamster S9	With 30% Rat S9	With 30% Rat S9
TA100	0	116 ± 6	111 ± 9	127 ± 6	151 ± 4	128 ± 6
	10			127 ± 1		
	33			155 ± 13		
	100	130 ± 2	220 ± 8	231 ± 18	153 ± 7	
	166			362 ± 13		
	333	137 ± 4	461 ± 32	496 ± 18	168 ± 10	159 ± 6
	666					180 ± 8
	1,000	114 ± 9	1,254 ± 8		294 ± 4	251 ± 19
	1,666					358 ± 17
	3,333	126 ± 5	1,146 ± 24		557 ± 21	554 ± 20
	6,666	117 ± 1				
	10,000		Toxic		157 ± 14[b]	
Trial summary		Negative	Positive	Positive	Positive	Positive
Positive control[c]		850 ± 20	645 ± 18	661 ± 19	541 ± 19	626 ± 13
TA98	0	22 ± 2	21 ± 2	24 ± 3	16 ± 3	22 ± 2
	100	20 ± 1	27 ± 1	30 ± 1	21 ± 4	
	166			45 ± 2		
	333	19 ± 3	57 ± 7	71 ± 2	19 ± 3	25 ± 2
	666			106 ± 4		34 ± 3
	1,000	22 ± 1	256 ± 6	251 ± 20	43 ± 3	48 ± 2
	1,666					52 ± 1
	3,333	21 ± 4	286 ± 4		68 ± 3	64 ± 3
	6,666	17 ± 1				
	10,000		Toxic		6 ± 1[d]	
Trial summary		Negative	Positive	Positive	Positive	Positive
Positive control		421 ± 28	311 ± 6	457 ± 15	251 ± 13	410 ± 6

[a]Study was performed at SRI International. The detailed protocol is presented by Zeiger et al.[47]. Data are presented as revertants/plate (mean ± standard error) from three plates. 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 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with both strains was 2-aminoanthracene.
[d]Slight toxicity and precipitate on plate.

Table E-2. Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of *o*-Chloropyridine in Drinking Water for Three Months[a]

	Exposure Concentration (ppm)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Water[d]	0	5	4.00 ± 0.35		3.92 ± 0.35
o-Chloropyridine	10	5	3.90 ± 0.60	0.5449	4.34 ± 0.55
	30	5	3.80 ± 0.37	0.5897	3.66 ± 0.46
	100	5	3.10 ± 0.33	0.8577	3.20 ± 0.28
	300	5	2.70 ± 0.41	0.9442	3.56 ± 0.19
	1,000	5	2.70 ± 0.20	0.9442	3.80 ± 0.22
			P = 0.959[e]		
Female					
Water	0	5	2.40 ± 0.24		3.52 ± 0.57
o-Chloropyridine	10	5	1.60 ± 0.29	0.8973	3.72 ± 0.23
	30	5	2.90 ± 0.64	0.2458	4.54 ± 0.39
	100	5	1.50 ± 0.32	0.9254	3.94 ± 0.53
	300	5	2.60 ± 0.29	0.3885	3.88 ± 0.56
	1,000	5	2.10 ± 0.48	0.6728	3.50 ± 0.24
			P = 0.511		

[a]Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al.[48]. NCE = normochromatic erythrocyte; PCE = polychromatic erythrocyte.
[b]Mean ± standard error.
[c]Pairwise comparison with the untreated control group; exposed group values are significant at $P \leq 0.005$.
[d]Untreated control.
[e]Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test; significant at $P \leq 0.025$.

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F.1. Procurement and Characterization

F.1.1. *o*-Chloropyridine

o-Chloropyridine was obtained from Aldrich Chemical Company (Milwaukee, WI) in one lot (15306CN) that was used in the 2-week dermal and 3-month drinking water studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH, and Richland, WA), Chemir/Polytech Laboratories, Inc. (Maryland Heights, MO), Galbraith Laboratories, Inc. (Knoxville, TN), and the study laboratory, BioReliance Corporation (Rockville, MD). Reports on analyses performed in support of the *o*-chloropyridine studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear colorless liquid, was identified as *o*-chloropyridine by the analytical chemistry laboratory, Chemir/Polytech Laboratories, Inc., and the study laboratory using infrared (IR) spectroscopy and by the analytical chemistry laboratory and Chemir/Polytech Laboratories, Inc., using proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the literature spectra[55; 56] of *o*-chloropyridine. The IR and NMR spectra are presented in Figure F-1, Figure F-2, and Figure F-3.

The purity of lot 15306CN was determined by Galbraith Laboratories, Inc., using elemental analyses and by the analytical chemistry laboratory using gas chromatography (GC). Elemental analyses for carbon, hydrogen, nitrogen, and chlorine were in agreement with the theoretical values for *o*-chloropyridine. GC using system A (Table F-1) indicated one major peak and two impurities with areas at least 0.1% relative to the major peak area; the purity of the bulk chemical was determined to be greater than 99%. GC using system B indicated one major peak and two impurity peaks with areas at least 0.1% of the major peak area; the purity of the test chemical was determined to be greater than 99.5%. The overall purity of lot 15306CN was determined to be 99% or greater.

To ensure stability, the bulk chemical was stored at room temperature in sealed amber glass bottles under a headspace of inert gas, protected from light and moisture. Periodic reanalyses of the bulk chemical were performed during the 2-week and 3-month studies by the study laboratory using GC by system C, and no degradation of the bulk chemical was detected.

F.1.2. Ethanol

Ninety-five percent ethanol was obtained from Clear Spring Distilling Company (Clermont, KY) in one lot (21049312) that was used as the dosing vehicle in the 2-week dermal studies. Lot 21049312, a clear liquid, was identified as ethanol by the study laboratory using IR spectroscopy; the IR spectrum was consistent with a literature spectrum[57] of ethanol.

The purity of lot 21049312 was determined by the study laboratory using GC by system D (Table F-1). Analysis indicated one major peak and three impurities each with a relative concentration of less than or equal to 0.0001%.

F.2. Preparation and Analysis of Dose Formulations

F.2.1. Dermal Studies

The dose formulations were prepared once during the 2-week studies by mixing *o*-chloropyridine with 95% ethanol to give the required concentrations (Table F-2). The dose formulations were stored under a headspace of inert gas in refrigerated amber glass vials with Teflon®-lined lids for up to 23 days.

Stability studies of a 3.125 mg/mL dose formulation were performed by the analytical chemistry laboratory with GC by system E (Table F-1). Stability was confirmed for at least 43 days for dose formulations stored in sealed amber glass vials at room temperature, 5°C, or −20°C. Data from a simulated animal room stability study indicated that *o*-chloropyridine was stable dissolved in 95% ethanol when exposed to light for up to 3 hours at room temperature.

The dose formulations were analyzed on the day they were prepared by the study laboratory using GC by system C. All seven dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table F-3). Animal room samples of these dose formulations were also analyzed; one of five formulations for rats and all five formulations for mice were within 10% of the target concentrations. High concentrations measured for some of the rat animal room samples were attributed to improper sealing of the vials after dosing and possible solvent evaporation.

F.2.2. Drinking Water Studies

The dose formulations were prepared nine times during the 3-month studies by mixing *o*-chloropyridine with tap water to give the required concentrations (Table F-2). The dose formulations were stored refrigerated in Cubitainers® with taps, protected from light, for up to 21 days.

Because all dose formulations in these studies were determined to be solutions, no homogeneity studies were required. Stability studies of the 10 ppm dose formulation were performed by the analytical chemistry laboratory with high-performance liquid chromatography (HPLC). The analytical system consisted of a Waters (Waters Corporation, Milford, MA) or Spectra-Physics (Spectra-Physics, Inc., Mountain View, CA) liquid chromatograph, a Luna® (5 µm particle size, C18 150 mm × 4.6 mm) column (Phenomenex, Inc., Torrance, CA), an isocratic mobile phase of acetonitrile:Milli-Q® water:glacial acetic acid (33:66:1) at a flow rate of 1.0 mL/minute, and ultraviolet detection at 254 nm. Stability was confirmed for at least 43 days for dose formulations stored in sealed polyethylene bottles protected from light at 5°C and for at least 8 days under simulated animal room conditions.

The dose formulations were analyzed at the beginning, midpoint, and end of the studies by the study laboratory using the HPLC system described above for the dose formulation stability studies; animal room samples of these dose formulations were also analyzed. All 15 dose formulations analyzed and used for rats and mice were within 10% of the target concentrations (Table F-4). Of the animal room samples analyzed, all 15 for rats and 12 of 15 for mice were within 10% of the target concentrations, and the remaining three were less than 12% of the target concentrations.

Table F-1. Gas Chromatography Systems Used in the Studies of *o*-Chloropyridine[a]

Detection System	Column	Oven Temperature Program
System A		
Flame ionization	Stabilwax®, 30 m × 0.53 mm, 1.0 µm film (Restek, Bellefonte, PA)	65°C for 1 minute, then 5°C/minute to 230°C, held for 1 minute
System B		
Flame ionization	Stabilwax®-DB, 30 m × 0.25 mm, 0.5 µm film (Restek)	50°C for 1 minute, then 10°C/minute to 200°C, held for 13 minutes
System C		
Flame ionization	DB™-WAX, 30 m × 0.53 mm, 1.0 µm film (J&W Scientific, Folsom, CA)	75°C for 1 minute, then 7°C/minute to 180°C, then 15°C/minute to 230°C, held for 1 minute
System D		
Flame ionization	DB™-WAX, 30 m × 0.53 mm, 1.0 µm film, J&W Scientific	40°C for 5 minutes, then 10°C/minute to 220°C, held for 5 minutes
System E		
Flame ionization	Stabilwax®-DB, 30 m × 0.25 mm, 0.5 µm film (Restek)	75°C for 1 minute, then 7°C/minute to 180°C, then 15°C/minute to 230°C, held for 1 minute

[a]All gas chromatographs were manufactured by Hewlett-Packard, (Palo Alto, CA).

Table F-2. Preparation and Storage of Dose Formulations in the Studies of *o*-Chloropyridine

Two-week Dermal Studies	Three-month Drinking Water Studies
Preparation	
Each dose formulation was prepared by diluting a weighed amount of *o*-chloropyridine with a specified volume of 95% ethanol in a graduated cylinder. The dose formulations were stirred on a magnetic stirrer until the mixtures were homogenized. Dose formulations were prepared once during the studies.	Tap water was weighed to a volume of 1 L less than the final volume of the formulation into a Cubitainer®. Water was removed from the Cubitainer® to account for the volume of *o*-chloropyridine to be added. *o*-Chloropyridine was weighed into a tared glass beaker and the contents were quantitatively transferred to the Cubitainer® using 1 L of tap water measured in a beaker. The final mixture was agitated by vigorous shaking. Dose formulations were prepared nine times.
Chemical Lot Number	
15306CN	15306CN
Maximum Storage Time	
23 days	21 days
Storage Conditions	
The dose formulations were stored in amber glass vials under a headspace of inert gas; the vials were sealed with Teflon®-lined septa and crimped aluminum caps and were stored refrigerated.	Dose formulations were stored in 20 L Cubitainers® with taps, refrigerated and protected from light.
Study Laboratory	
BioReliance Corporation (Rockville, MD)	BioReliance Corporation (Rockville, MD)

Table F-3. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Two-week Dermal Studies of *o*-Chloropyridine

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	Difference from Target (%)
September 12, 2000	September 12, 2000	3.13	3.20	+7
		6.25	6.31	+1
		12.5	12.4	−1
		25	25.1	0
		50	49.0	−2
		100	98.0	−2
		200	194	−3
	October 11, 2000[c]	12.5	13.5	+8
		25	29.2	+17
		50	55.3	+11
		100	111	+11
		200	236	+18
	October 11, 2000[d]	3.13	3.22	+3
		6.25	6.38	+2
		12.5	12.9	+3
		25	26.5	+6
		50	53.1	+6

[a]The 3.13 and 6.25 mg/mL dose formulations were used only for mice, and the 100 and 200 mg/mL dose formulations were used only for rats.
[b]Results of duplicate analyses. For rats, dosing volume = 0.5 mL/kg; 12.5 mg/mL = 6.25 mg/kg, 25 mg/mL = 12.5 mg/kg, 50 mg/mL = 25 mg/kg; 100 mg/mL = 50 mg/kg; 200 mg/mL = 100 mg/kg. For mice, dosing volume = 2 mL/kg; 3.13 mg/mL = 6.25 mg/kg, 6.25 mg/mL = 12.5 mg/kg, 12.5 mg/mL = 25 mg/kg; 25 mg/mL = 50 mg/kg, 50 mg/mL = 100 mg/kg.
[c]Animal room samples for rats.
[d]Animal room samples for mice.

Table F-4. Results of Analyses of Dose Formulations Administered to Rats and Mice in the Three-month Drinking Water Studies of *o*-Chloropyridine

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
July 22, 2003	July 22, 2003	10	9.76	−2
		30	29.7	−1
		100	97.9	−2
		300	300	0
		1,000	897[b]	−10
	August 7, 2003[c]	10	9.23	−8
		30	27.9	−7
		100	94.2	−6
		300	278	−7
	August 7, 2003[d]	10	8.79	−12
		30	26.7	−11
		100	93.0	−7
		300	270	−10
July 25, 2003	July 25, 2003	1,000	982[e]	−2
	August 7, 2003[c]	1,000	936	−6
	August 7, 2003[d]	1,000	905	−10
August 25, 2003	August 26, 2003	10	9.92	−1
		30	28.8	−4
		100	98.1	−2
		300	296	−1
		1,000	992	−1
	September 17, 2003[c]	10	9.33	−7
		30	28.0	−7
		100	92.0	−8
		300	282	−6
		1,000	944	−6
	September 17, 2003[d]	10	9.16	−8
		30	27.1	−10
		100	92.1	−8
		300	279	−7
		1,000	889	−11
October 14, 2003	October 16, 2003	10	9.54	−5
		30	28.8	−4

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
		100	98.0	−2
		300	290	−3
		1,000	974	−3
	November 5, 2003[c]	10	9.59	−4
		30	28.3	−6
		100	94.6	−5
		300	278	−7
		1,000	930	−7
	November 5, 2003[d]	10	9.31	−7
		30	28.0	−7
		100	95.1	−5
		300	269	−10
		1,000	914	−9

[a]Results of duplicate analyses.
[b]Remixed; not used in studies.
[c]Animal room samples for rats.
[d]Animal room samples for mice.
[e]Results of remix.

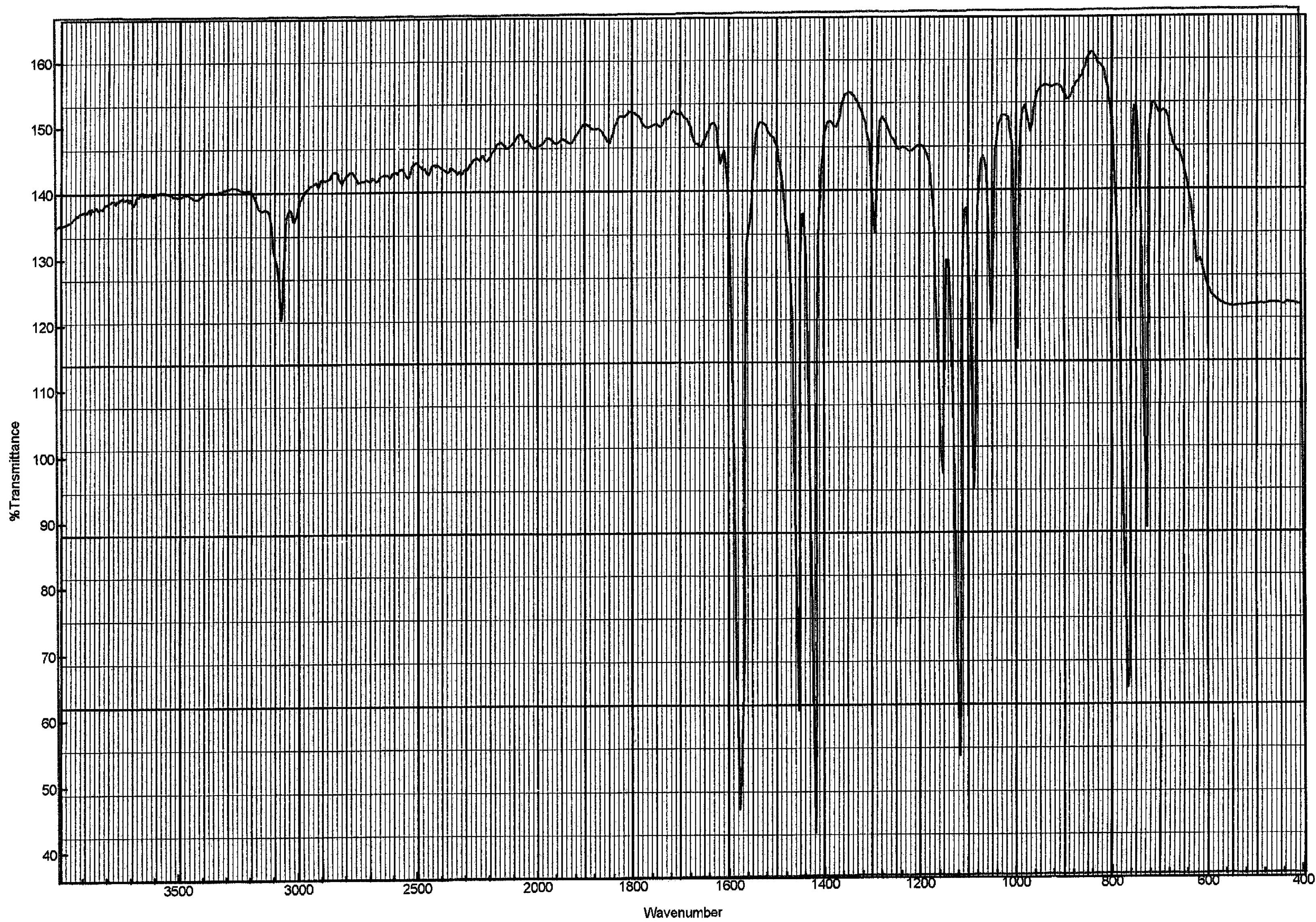

Figure F-1. Infrared Absorption Spectrum of *o*-Chloropyridine

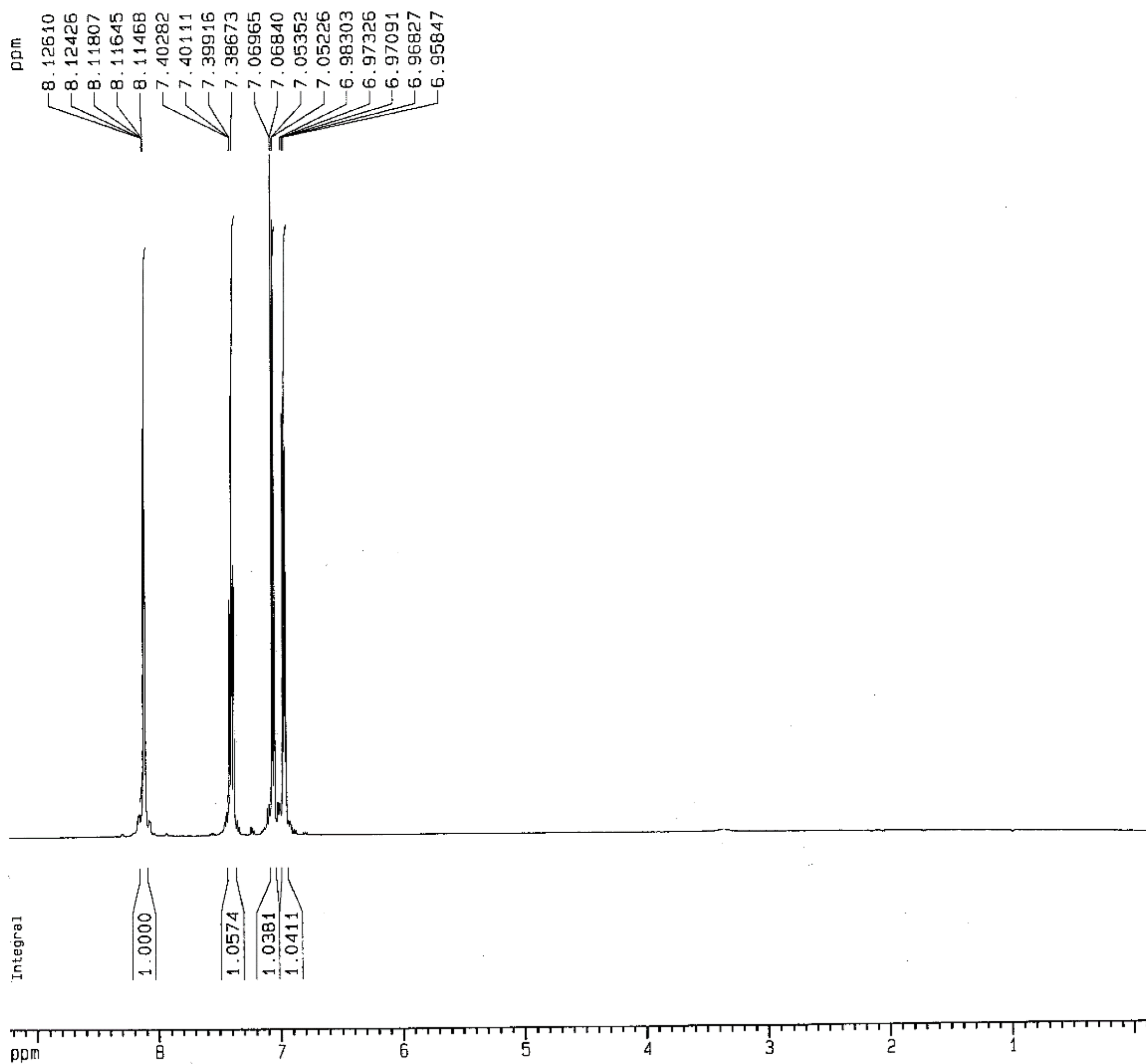

Figure F-2. Proton Nuclear Magnetic Resonance Spectrum of *o*-Chloropyridine

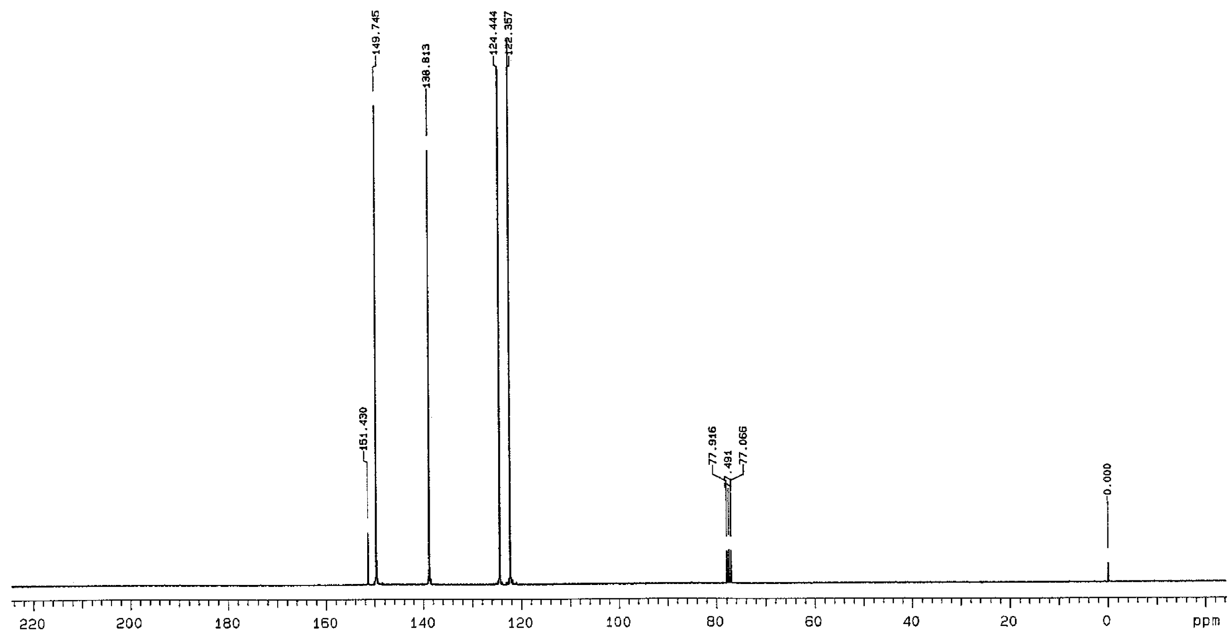

Figure F-3 Carbon-13 Nuclear Magnetic Resonance Spectrum of *o*-Chloropyridine

Appendix G. Water and Compound Consumption in the Three-month Drinking Water Studies of *o*-Chloropyridine

Tables

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Table G-2. Water and Compound Consumption by Female Rats in the Three-month Drinking Water Study of *o*-Chloropyridine G-3
Table G-3. Water and Compound Consumption by Male Mice in the Three-month Drinking Water Study of *o*-Chloropyridine G-4
Table G-4. Water and Compound Consumption by Female Mice in the Three-month Drinking Water Study of *o*-Chloropyridine G-5

Table G-1. Water and Compound Consumption by Male Rats in the Three-month Drinking Water Study of *o*-Chloropyridine

Week	0 ppm		10 ppm			30 ppm			100 ppm		
	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	14.7	90	15.5	93	1.7	15.1	92	4.9	15.4	91	16.9
2	14.9	119	16.6	123	1.3	15.8	123	3.8	15.4	120	12.9
3	16.6	146	17.7	152	1.2	18.3	152	3.6	15.7	148	10.6
4	17.2	175	18.3	182	1.0	18.8	184	3.1	16.6	174	9.6
5	17.3	199	17.6	205	0.9	18.4	209	2.6	18.8	195	9.6
6	15.7	217	16.6	225	0.7	17.2	230	2.2	20.2	213	9.5
7	16.5	234	19.3	239	0.8	18.9	247	2.3	16.8	233	7.2
8	16.2	247	17.1	254	0.7	21.7	263	2.5	17.1	247	6.9
9	16.4	262	16.5	268	0.6	18.0	278	1.9	18.0	260	6.9
10	16.0	275	15.9	281	0.6	19.0	295	1.9	19.5	277	7.0
11	16.3	285	16.0	291	0.6	17.3	305	1.7	19.7	289	6.8
12	16.9	297	17.0	302	0.6	17.3	316	1.6	19.6	305	6.4
13	16.8	306	16.2	311	0.5	18.8	324	1.7	18.1	312	5.8
14	17.3	320	17.7	326	0.5	13.6	326	1.3	18.1	326	5.6

Week	300 ppm			1,000 ppm		
	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	13.6	91	44.9	6.5	94	68.9
2	15.2	118	38.6	8.8	98	89.6
3	16.6	145	34.3	10.2	121	84.5
4	16.9	172	29.5	11.3	145	77.9
5	16.5	192	25.8	12.0	164	73.0
6	15.2	210	21.7	11.3	182	62.1
7	17.7	222	24.0	11.7	195	60.0
8	16.9	239	21.2	11.6	209	55.4
9	16.0	255	18.9	12.8	222	57.7
10	15.8	267	17.8	13.0	219	59.4
11	16.1	275	17.6	12.7	235	54.0
12	16.4	286	17.2	12.8	249	51.4
13	16.1	293	16.5	13.0	258	50.4
14	17.5	309	17.0	15.1	268	56.4

[a]Grams of water consumed per animal per day.
[b]Milligrams of *o*-chloropyridine consumed per kilogram body weight per day.

Table G-2. Water and Compound Consumption by Female Rats in the Three-month Drinking Water Study of *o*-Chloropyridine

Week	0 ppm		10 ppm			30 ppm			100 ppm		
	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	12.5	86	13.8	88	1.6	13.0	87	4.5	12.6	88	14.3
2	13.4	106	13.8	108	1.3	13.6	108	3.8	13.4	108	12.5
3	13.9	120	14.1	122	1.2	15.8	123	3.8	13.5	122	11.1
4	14.4	135	14.8	137	1.1	15.5	139	3.3	13.8	137	10.1
5	15.5	146	15.7	149	1.1	15.7	150	3.1	12.4	149	8.3
6	14.0	158	15.7	161	1.0	14.9	160	2.8	15.2	161	9.5
7	14.0	168	14.4	171	0.8	16.2	169	2.9	13.8	170	8.1
8	13.5	175	14.0	177	0.8	16.3	176	2.8	13.3	176	7.6
9	12.4	179	13.4	181	0.7	13.8	180	2.3	12.9	179	7.2
10	13.0	184	13.6	185	0.7	14.4	185	2.3	12.7	183	6.9
11	14.3	188	13.5	190	0.7	14.6	190	2.3	14.5	189	7.7
12	14.2	191	13.9	194	0.7	14.1	195	2.2	14.5	192	7.6
13	14.1	192	13.8	196	0.7	15.2	195	2.3	12.8	194	6.6
14	13.4	195	17.7	200	0.9	14.8	200	2.2	17.4	196	8.9

Week	300 ppm			1,000 ppm		
	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	12.2	86	42.6	6.1	87	70.1
2	13.1	105	37.6	7.7	89	86.1
3	13.3	119	33.7	8.7	105	83.1
4	13.6	132	30.9	9.4	119	78.9
5	13.7	145	28.4	10.0	130	77.0
6	13.1	155	25.4	10.0	139	71.9
7	12.7	163	23.4	9.8	148	66.4
8	12.8	168	22.8	9.8	154	63.7
9	12.2	173	21.2	9.8	158	62.0
10	12.6	176	21.5	10.1	163	61.9
11	12.2	182	20.1	10.3	167	61.6
12	12.3	185	20.0	10.5	170	61.6
13	13.0	187	20.8	10.4	173	60.2
14	12.7	190	20.0	15.8	174	90.9

[a]Grams of water consumed per animal per day.
[b]Milligrams of *o*-chloropyridine consumed per kilogram body weight per day.

Table G-3. Water and Compound Consumption by Male Mice in the Three-month Drinking Water Study of *o*-Chloropyridine

	0 ppm		10 ppm			30 ppm			100 ppm		
Week	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	4.8	23.2	5.1	23.2	2.2	5.4	23.3	7.0	5.3	23.3	22.7
2	4.9	25.9	5.2	26.2	2.0	5.4	26.2	6.2	5.1	26.0	19.6
3	4.6	27.5	4.8	27.6	1.7	5.0	28.1	5.3	4.5	27.7	16.2
4	4.5	29.0	4.8	29.4	1.6	4.5	29.9	4.5	4.5	29.4	15.3
5	4.9	30.5	5.1	31.1	1.6	5.0	31.6	4.7	4.7	30.9	15.2
6	4.3	31.9	4.6	32.9	1.4	4.7	33.3	4.2	4.8	32.1	15.0
7	4.8	33.9	4.8	34.4	1.4	4.9	34.8	4.2	4.5	33.4	13.5
8	5.1	35.4	5.1	36.2	1.4	4.9	36.4	4.0	5.1	34.8	14.7
9	4.1	36.5	4.8	37.4	1.3	4.8	37.6	3.8	4.8	36.1	13.3
10	4.6	37.5	4.5	38.4	1.2	4.6	38.6	3.6	4.5	37.1	12.1
11	4.7	37.5	4.8	38.9	1.2	4.4	38.7	3.4	4.5	37.5	12.0
12	5.0	38.8	5.1	40.3	1.3	4.9	40.1	3.7	5.0	38.2	13.1
13	5.1	40.1	5.2	41.7	1.2	5.2	41.2	3.8	4.8	39.3	12.2
14	4.5	40.8	4.6	42.4	1.1	4.9	42.4	3.5	5.4	40.2	13.4

	300 ppm			1,000 ppm		
Week	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	4.4	23.1	57.1	3.2	23.1	138.3
2	4.5	25.9	52.1	2.8	24.6	113.8
3	4.4	27.8	47.4	3.0	26.0	115.4
4	4.3	29.4	43.9	2.9	26.8	108.4
5	4.4	30.8	42.8	3.2	27.7	115.5
6	4.2	32.5	38.8	3.2	28.8	111.3
7	4.2	34.0	37.1	3.3	29.7	111.1
8	4.7	35.6	39.6	3.4	30.5	111.6
9	4.3	36.9	34.9	3.3	31.0	106.6
10	4.2	37.7	33.4	3.2	31.4	101.9
11	4.1	37.8	32.5	3.0	31.5	95.4
12	4.3	38.7	33.3	3.3	32.0	103.0
13	4.9	40.0	36.7	3.4	32.5	104.6
14	4.7	40.5	34.8	3.3	33.0	100.1

[a]Grams of water consumed per animal per day.
[b]Milligrams of *o*-chloropyridine consumed per kilogram body weight per day.

Table G-4. Water and Compound Consumption by Female Mice in the Three-month Drinking Water Study of *o*-Chloropyridine

	0 ppm		10 ppm			30 ppm			100 ppm		
Week	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	2.8	18.6	3.0	19.1	1.6	2.7	18.8	4.3	2.3	19.0	12.1
2	3.1	19.9	2.9	20.6	1.4	2.9	20.0	4.3	2.7	19.7	13.7
3	2.9	19.7	2.5	20.6	1.2	3.0	20.6	4.4	2.9	20.3	14.3
4	3.3	21.4	2.6	19.5	1.3	2.5	21.4	3.5	2.8	21.5	13.0
5	3.7	23.3	3.7	23.1	1.6	3.4	22.6	4.5	3.0	22.8	13.1
6	3.0	23.2	3.1	23.3	1.3	3.0	23.8	3.8	2.5	23.0	10.9
7	3.1	25.2	3.5	25.4	1.4	3.1	25.5	3.7	3.1	25.4	12.2
8	2.8	25.6	3.1	26.5	1.2	2.8	26.1	3.2	2.5	26.3	9.5
9	3.3	27.4	3.4	27.0	1.3	3.4	26.3	3.9	3.0	27.3	11.0
10	3.1	27.0	3.2	27.7	1.2	3.3	27.7	3.6	3.1	27.8	11.1
11	3.0	28.3	3.3	28.6	1.2	3.1	28.5	3.3	3.1	28.6	10.9
12	3.7	29.3	3.7	29.2	1.3	3.2	29.5	3.3	3.1	29.7	10.4
13	3.0	29.2	4.3	30.1	1.4	2.4	30.6	2.4	2.6	29.6	8.8
14	3.5	28.5	3.3	31.8	1.0	3.4	31.2	3.3	3.4	30.3	11.2

	300 ppm			1,000 ppm		
Week	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	3.3	18.1	54.7	1.6	18.5	86.3
2	2.7	20.3	40.0	3.8	18.9	200.3
3	3.3	22.1	44.7	1.4	18.9	74.0
4	2.6	22.3	34.9	1.1	21.0	52.4
5	3.7	23.9	46.4	1.3	23.0	56.5
6	2.9	25.0	34.8	2.4	22.9	104.7
7	2.9	26.4	33.0	2.1	24.7	85.1
8	2.7	26.6	30.5	2.2	25.2	87.3
9	3.2	28.4	33.8	2.3	25.8	89.3
10	3.3	29.3	33.8	2.6	26.8	96.9
11	4.3	30.0	43.0	2.5	27.5	91.0
12	3.4	31.3	32.6	2.9	28.3	102.6
13	3.2	31.2	30.8	1.8	27.6	65.2
14	3.4	31.9	32.0	2.8	28.4	98.6

[a]Grams of water consumed per animal per day.
[b]Milligrams of *o*-chloropyridine consumed per kilogram body weight per day.

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

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Table H-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 H-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
B12	5 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 H-3. Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	15.2	15.2	1
Crude fat (% by weight)	8.0	8.0	1
Crude fiber (% by weight)	943	9.4	1
Ash (% by weight)	5.3	5.3	1
Amino Acids (% of total diet)			
Arginine	0.783 ± 0.070	0.670–0.970	22
Cystine	0.220 ± 0.024	0.150–0.250	22
Glycine	0.701 ± 0.041	0.620–0.800	22
Histidine	0.352 ± 0.077	0.270–0.680	22
Isoleucine	0.546 ± 0.044	0.430–0.660	22
Leucine	1.095 ± 0.067	0.960–1.240	22
Lysine	0.711 ± 0.114	0.310–0.860	22
Methionine	0.409 ± 0.046	0.260–0.490	22
Phenylalanine	0.628 ± 0.040	0.540–0.720	22
Threonine	0.505 ± 0.043	0.430–0.610	22
Tryptophan	0.150 ± 0.028	0.110–0.200	22
Tyrosine	0.401 ± 0.061	0.280–0.540	22
Valine	0.665 ± 0.043	0.550–0.730	22
Essential Fatty Acids (% of total diet)			
Linoleic	3.95 ± 0.259	3.49–4.55	22
Linolenic	0.30 ± 0.032	0.21–0.35	22
Vitamins			
Vitamin A (IU/kg)	6,920	6,920	1
Vitamin D (IU/kg)	1,000 [a]	–	–
α-Tocopherol (ppm)	80.6 ± 22.03	27.0–124.0	22
Thiamine (ppm)[b]	8.8	8.8	1
Riboflavin (ppm)	7.6 ± 2.89	4.20–17.50	22
Niacin (ppm)	78.9 ± 9.08	66.4–98.2	22
Pantothenic acid (ppm)	26.9 ± 12.63	17.4–81.0	22
Pyridoxine (ppm)[b]	9.54 ± 1.99	6.44–13.7	22
Folic acid (ppm)	1.62 ± 0.48	1.15–3.27	22
Biotin (ppm)	0.32 ± 0.10	0.20–0.704	22
Vitamin B_{12} (ppb)	53.6 ± 39.6	18.3–174.0	22
Choline (ppm)[b]	2,846 ± 485	1,820–3,790	22

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Minerals			
Calcium (%)	0.959	0.959	1
Phosphorus (%)	0.589	0.589	1
Potassium (%)	0.666 ± 0.030	0.626–0.733	22
Chloride (%)	0.386 ± 0.039	0.300–0.474	22
Sodium (%)	0.189 ± 0.016	0.160–0.222	22
Magnesium (%)	0.216 ± 0.062	0.185–0.490	22
Sulfur (%)	0.170 ± 0.029	0.116–0.209	14
Iron (ppm)	186 ± 39.2	135–311	22
Manganese (ppm)	51.4 ± 10.28	21.0–73.1	22
Zinc (ppm)	53.4 ± 8.46	43.3–78.5	22
Copper (ppm)	7.01 ± 2.562	3.21–16.3	22
Iodine (ppm)	0.503 ± 0.206	0.158–0.972	22
Chromium (ppm)	0.694 ± 0.276	0.330–1.380	22
Cobalt (ppm)	0.256 ± 0.164	0.098–0.864	22

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

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

	Mean[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.50	0.50	1
Cadmium (ppm)	0.08	0.08	1
Lead (ppm)	0.05	0.05	1
Mercury (ppm)	<0.02	–	1
Selenium (ppm)	0.16	016	1
Aflatoxins (ppb)	<5.00	–	1
Nitrate nitrogen (ppm)[c]	17.7	17.7	1
Nitrite nitrogen (ppm)[c]	<0.61	–	1
BHA (ppm)[d]	<1.0	–	1
BHT (ppm)[d]	<1.0	–	1
Aerobic plate count (CFU/g)	10	10	1
Coliform (MPN/g)	3.0	3.0	1
Escherichia coli (MPN/g)	<10	–	1
Salmonella (MPN/g)	Negative	–	1
Total nitrosoamines (ppb)[e]	3.5	3.5	1
N-Nitrosodimethylamine (ppb)[e]	1.8	1.8	1
N-Nitrosopyrrolidine (ppb)[e]	1.7	1.7	1
Pesticides (ppm)			
α-BHC	<0.01	–	1
β-BHC	<0.02	–	1
γ-BHC	<0.01	–	1
δ-BHC	<0.01	–	1
Heptachlor	<0.01	–	1
Aldrin	<0.01	–	1
Heptachlor epoxide	<0.01	–	
DDE	<0.01	–	1
DDD	<0.01	–	1
DDT	<0.01	–	1
HCB	<0.01	–	1
Mirex	<0.01	–	1
Methoxychlor	<0.05	–	1
Dieldrin	<0.01	–	1
Endrin	<0.01	–	1
Telodrin	<0.01	–	1

	Mean[b]	**Range**	**Number of Samples**
Chlordane	<0.05	–	1
Toxaphene	<0.10	–	1
Estimated PCBs	<0.20	–	1
Ronnel	<0.01	–	1
Ethion	<0.02	–	1
Trithion	<0.05	–	1
Diazinon	<0.10	–	1
Methyl chlorpyrifos	0.175	0.175	1
Methyl parathion	<0.02	–	1
Ethyl parathion	<0.02	–	1
Malathion	0.589	0.589	1
Endosulfan I	<0.01	–	1
Endosulfan II	<0.01	–	1
Endosulfan sulfate	<0.03	–	1

[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 I. Sentinel Animal Program

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I.1. Methods

Rodents used in 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 test compounds. Under this program, the disease state of the rodents is monitored via sera from extra (sentinel) or dosed animals in the study rooms. The sentinel animals and the dosed animals are subject to identical environmental conditions. Furthermore, the sentinel animals come from the same production source and weanling groups as the animals used for the studies of test compounds.

For the 3-month studies, blood samples were collected, allowed to clot, and the serum was separated. The serum samples were processed appropriately at BioReliance Corporation (Rockville, MD) for determination of the presence of pathogens. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

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

Method and Test	Time of Collection
Rats	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
Mice	
ELISA	
Ectromelia virus	Study termination
EDIM (epizootic diarrhea of infant mice)	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
MAd-FL (Mouse adenoma virus-)1	Study termination
MHV (mouse hepatitis virus)	Study termination
PVM	Study termination
Reovirus	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination

I.2. Results

All results were negative.

Appendix J. Absorption, Distribution, Metabolism, Excretion, and Toxicokinetics

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J.1. Introduction

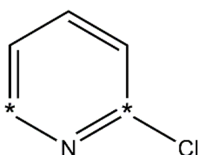
The objective of these studies was to determine the disposition and metabolism of *o*-chloropyridine in male F344 rats after a single oral gavage administration of 0.1 or 10 mg/kg of *o*-chloropyridine. Limited studies were also conducted following intravenous and intraperitoneal dosing. Concentration of parent compound or the total radioactivity was also determined in blood in order to establish basic toxicokinetic parameters. Attempts were also made to identify metabolites of *o*-chloropyridine in rat urine and bile after a single oral or intraperitoneal dose.

J.2. Materials and Methods

J.2.1. Chemicals

Nonradiolabeled *o*-chloropyridine (CAS No. 109-09-1, purity 99%, lot 03721) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). The identity of nonradiolabeled *o*-chloropyridine was confirmed by mass spectrometry (MS). The position of the chloro substituent was confirmed by chromatographic resolution of isomeric mono- and dichloropyridine standards using high-performance liquid chromatography (HPLC).

o-Chloropyridine radiolabeled with ^{14}C in the 2 and 6 positions (lot 3228-292, specific activity 58.50 mCi/mmol), in an ethanol solution was received from New England Nuclear Corporation (Boston, MA).



Structure of [^{14}C]-*o*-chloropyridine
(*denotes position of ^{14}C)

The radiochemical purity of the test material was determined to be equal to or higher than 95% by HPLC.

Prospective metabolites of *o*-chloropyridine, N-methylpyridine-2-mercapturic acid, pyridine-N-oxide-2-mercapturic acid, 2-methylthiopyridine, 2-methylthiopyridine-N-oxide, 2-chloro-5-pyridinol, and 2-chloro-4-pyridinol were synthesized in-house using methods of Talik[58], Beak and Lee[59], Westland et al.[60], and Kohrman et al.[61].

J.2.2. Animals

Young adult male F344 rats were obtained from Charles River Laboratories, Inc. (Raleigh, NC), and were quarantined for at least 1 week prior to the start of the study. Animals were fed certified Purina Rodent Chow (5002; Purina Mills, Inc., St. Louis, MO) and furnished tap water ad libitum. During acclimation (1 day prior to dosing) and following dosing, metabolism study animals were housed individually in metabolism chambers that provided for separate collection of urine, feces, and volatiles. Biliary excretion study rats were kept on heating pads to maintain body temperature throughout the study. Toxicokinetic study rats were housed individually in

cages following surgical implantation of the jugular cannulae and throughout the toxicokinetic study. In the animal rooms, air circulation was 100% fresh filtered, room temperature was maintained at 64° to 79°F, and relative humidity ranged from 30% to 70%. Light and darkness were cycled at 12-hour intervals.

Anesthesia was used to avoid undue pain or distress. Rats were anesthetized with ketamine/xylazine (7:1, 50 to 80 mg/kg intramuscular or to effect) prior to surgical implantation of the jugular cannula[62] for toxicokinetic studies. Biliary excretion study rats were anesthetized with sodium pentobarbital, orally (35 mg/kg) and intraperitoneally (45 mg/kg), prior to cannulation of the bile duct. A state of anesthesia was maintained throughout the bile collection period by injection of sodium pentobarbital (5 mg/kg) as needed. At the end of the oral gavage studies, rats were euthanized by exsanguination and section of the diaphragm. Toxicokinetic and biliary excretion study rats were euthanized by CO_2 overexposure after the final collection.

J.2.3. Study Design

Absorption, distribution, metabolism, and excretion of *o*-chloropyridine were investigated in groups of rats following a single oral gavage dose of either 0.1 or 10 mg [^{14}C]-*o*-chloropyridine/kg body weight (10 µCi per animal) and killed 72 hours following dosing. Toxicokinetics of *o*-chloropyridine was investigated in jugular vein-cannulated rats receiving a single oral gavage dose of 10 mg/kg or a single intravenous injection of 1 mg/kg (30 µCi per animal in each case). Biliary excretion and metabolism were investigated in rats receiving a single intraperitoneal injection of 50 mg/kg (35 µCi per animal) for bile collection for up to 4 hours.

For all studies, formulations were prepared by addition of Alkamuls® EL-620/L (no more than 15% of the dose) (Rhodia, Inc., Cranbury, NJ) to known amounts of *o*-chloropyridine following which distilled deionized water and the appropriate amount of [^{14}C]-*o*-chloropyridine were added. Dosing volumes were either 5 mL/kg (oral gavage and intraperitoneal injection or 1 mL/kg (intravenous injection). Oral doses were administered by intragastic gavage using a syringe equipped with a 16-gauge gavage needle. Intravenous doses were injected into a lateral tail vein using a syringe equipped with a 27-gauge needle.

J.2.4. Sample Collection

In mass balance studies, urine and feces were collected separately into containers cooled with dry ice for up to 72 hours. At terminal kill, urine was collected directly from the bladder and added to the final urine collection. After terminal kill, the cages were rinsed well with water and ethanol. Urine, feces, and cage rinse collections were stored in the dark at −20°C until analyzed. Volatiles were collected by passing air from the metabolism chamber through a series of traps containing ethanol and 1 N NaOH for collection of volatile organics and CO_2, respectively.

At terminal kill, blood from anesthetized rats was collected into a heparinized syringe by cardiac puncture. Duplicate samples of skin (ears), triplicate samples of muscle (hind leg and abdominal) and adipose tissue (perirenal and epididymal), and the entire kidneys, liver, spleen, lungs, testes, bladder, heart, and brain were removed. The stomach, small intestine, cecum, and large intestine (all included contents) were removed and the carcasses were stored.

Bile was collected via a bile duct cannula extension for up to 4 hours postdosing. Bile collections were stored at −80°C prior to analysis.

In toxicokinetic studies, blood samples were collected from rats via a jugular cannula for up to 72 hours postdosing (oral gavage study) and for up to 4 hours postdosing (intravenous injection study). In the rat intravenous injection study, the volume of blood withdrawn at each time point was replaced with plasma obtained from donor animals. Blood samples in these studies were extracted immediately after collection as described below.

J.2.5. Sample Analysis

Whole blood samples (approximately 300 µL per animal per time point) from toxicokinetic studies were extracted three times with twice the volume of hexanes. The three extracts from each sample were pooled and stored at −20°C prior to analysis for total radioactivity and for parent *o*-chloropyridine (intravenous studies only) by HPLC as described below.

Radioactivity in samples was measured by liquid scintillation spectrophotometry (LSS). Ultima Gold™ scintillation cocktail (Packard Instrument Company, Meriden, CT) was used in all determinations of radiochemical content. Duplicate aliquots of urine, duplicate aliquots of bile, triplicate aliquots of cage rinse and trapping solutions from breath traps, and duplicate aliquots of blood extracts were directly analyzed. Triplicate samples of feces were weighed, homogenized with equal amounts of water, and solubilized in Soluene®-350 (Packard Instrument Company). Tissue samples (from large tissues) and small tissues and organs in their entirety were digested in Soluene®-350. Large organs like the liver were homogenized, and weighed aliquots of the homogenate were used. Blood samples were neutralized and decolorized by treatment with perchloric acid and H_2O_2 prior to solubilization. Gastrointestinal tract tissues and carcass were digested in 2 N ethanolic NaOH prior to analysis of triplicate aliquots for radioactivity.

J.2.6. Metabolic Profiling and Identification

Aliquots of the 1-hour and 4-hour bile collections were mixed with an equal amount of the initial-condition mobile phase containing 0.1% trifluoroacetic acid (TFA) and the mixture was centrifuged prior to analysis by HPLC (see below). An aliquot of bile was also incubated with β-glucuronidase type VII-A, obtained from *Escherichia coli* for 17 hours at 37°C, and centrifuged prior to HPLC analysis.

Composite urine samples (0 to 24 hours) from the 10 mg/kg oral gavage study were profiled using HPLC. Urine was subjected to acid hydrolysis to determine whether acid labile conjugates were present. Urine was hydrolyzed by addition of 1 N HCl followed by heating overnight between 50° and 55°C. After hydrolysis, the samples were neutralized and analyzed by HPLC/LSS.

Radiolabeled urinary metabolites were isolated using HPLC as described below. Fractions containing the same metabolites collected from a number of injections were pooled and concentrated. Other chromatographic conditions were then developed for the separation of the urinary metabolites in the fractions containing multiple radiolabeled components. Selected fractions were analyzed by MS and nuclear magnetic resonance (NMR) spectroscopy for identification of metabolites.

J.2.7. High-Performance Liquid Chromatography

The HPLC system consisted of two Waters pumps (models 515, 510, or 6000A; Waters Corporation, Milford, MA) or a Waters 600E solvent delivery system, a Rheodyne Model 7125 injector (Rheodyne, LLC, Rohnert Park, CA), an ABI 759A absorbance detector (set at 230 or 254 nm; Applied Biosystems, Inc., Foster City, CA) or a Waters 2487 dual absorbance detector (set at 250 and 285 nm), and a βRAM-LS radioactivity detector equipped with a 200-, 250-, or 500-µL lithium glass flowthrough solid scintillator flow cell. The flow rate was 1 mL/minute unless otherwise specified. Column effluent was collected in timed fractions and assayed for [^{14}C] content using LSS.

Blood extracts for *o*-chloropyridine levels were analyzed on an Alltech® Alltima™ Cyano 100-Å column (250 mm × 4.6 mm, 5 µm particle size; Alltech Associates, Inc., Deerfield, IL). The analysis was isocratic with a mobile phase consisting of 95:5 (v:v) hexanes:ethanol.

Profiling of urinary metabolites was done on a Phenomenex® Luna® C18 column (150 mm × 4.6 mm, 5 µm particle size; Phenomenex, Inc., Torrance, CA) using acetonitrile and water, both containing 0.1% TFA. The acetonitrile gradient was linearly increased as follows: 2% for 5 minutes, to 10% over 15 minutes, and then to 50% over 5 minutes. Acid-hydrolyzed urine samples were analyzed the same way except that the gradient was changed from 2% to 20% over 15 minutes.

Further profiling of the urinary metabolites and profiling of bile utilized a Phenomenex® Aqua® C18 column (250 mm × 4.6 mm, 5 µm particle size); the mobile phases used were 100% 0.05 M ammonium acetate buffer, pH 4, and acetonitrile. Urine was eluted with a linear gradient from 0% to 75% acetonitrile over 30 minutes. Bile was eluted with water and acetonitrile, both containing 0.1% TFA, at a flow rate of 2 mL/minute. The acetonitrile gradient was linearly increased as follows: 3% for 2 minutes, to 11% over 16 minutes, to 25% over 6 minutes, and then to 90% over 2 minutes. For the isolation of biliary metabolites, the same mobile phase gradient was run at a flow rate of 4 mL/minute on a Phenomenex® Aqua® C18 column (250 mm × 10 mm, 5 µm particle size).

Urinary metabolites were isolated using a Phenomenex® Aqua® C18 column (250 mm × 10 mm, 5 µm particle size) using acetonitrile and water, both containing 0.1% TFA. The acetonitrile gradient was linearly increased as follows: 3% for 5 minutes, to 10% over 20 minutes, to 15% over 5 minutes, and then to 90% over 2 minutes. The flow rate was 4 mL/minute. Variations of this method using isocratic conditions of 8%, 10%, 20%, or 25% acetonitrile were used to further purify the urine fractions as necessary.

Analysis of urinary fractions for metabolite identification by LC/MS used a Phenomenex® Aqua® C18 column (250 mm × 4.6 mm, 5 µm particle size). The mobile phases used were acetonitrile and water, both containing 0.1% TFA at isocratic conditions of 20%, 30%, 40%, or 60% acetonitrile and a flow rate of 0.5 mL/minute.

J.2.8. Other Instrumentation

NMR spectra were obtained using a Bruker® AMX-500 NMR spectrometer (Bruker BioSpin Corporation, Newark, DE) and a Bruker® AVANCE™-300 spectrometer. Mass spectra were obtained using a Hewlett Packard 6890 Series capillary gas chromatograph (Hewlett-Packard

Company, Palo Alto, CA) and a HP 5973 mass selective detector or on a PE SCIEX API 150 EX analyzer (Perkin Elmer Sciex, Wellesley, MA) attached to an Agilent 110 Series Tower (Agilent Technologies) with an IN/US PRAM (IN/US Systems, Inc., Tampa, FL) inline.

J.2.9. Toxicokinetic Analysis

Blood concentration-time data were analyzed by model-dependent methods using WinNonlin® version 1.0 (Pharsight Corporation, Mountain View, CA). Three different methods of weighting the data points (uniform, 1/y, and $1/y^2$) were utilized. A statistical F test was used for the selection of the appropriate number of compartments for the best-fit model. The two-compartment model resulted in the best fits to the blood *o*-chloropyridine concentration-time data in rats after intravenous injection. The data for one rat were poorly fitted by the simulation at the last two time points. For this reason, this rat was excluded from the analysis. The two-compartment model is described by the following equation:

$$C(t) = A \times e^{-\alpha t} + B \times e^{-\beta t}$$

where constants A and B are intercepts on the y axis for each exponential segment of the curve and β and α are the elimination and distribution rate constants, respectively.

J.3. Results and Discussion

J.3.1. Metabolism and Disposition of *o*-Chloropyridine

Disposition data for rats receiving a single oral gavage dose of 10 mg [^{14}C]-*o*-chloropyridine/kg body weight are presented in Table J-1. As shown in the table, 81.9% of the dose was recovered after 72 hours with 35.9% in the urine, 28.4% in the feces, and 12.5% and 1.14%, respectively, as CO_2 and as volatile organics. Tissue distribution of radiolabel in the same animals is shown in Table J-2. Approximately 0.5% and 3.4% of the dose was found in the residual carcass and selected tissues, respectively, at 72 hours after dosing. The liver and kidney were the only tissues that were found to have appreciable tissue:blood ratios. Data for rats receiving a single oral gavage dose of 0.1 mg/kg [^{14}C]-*o*-chloropyridine show that there are no dose-related differences in the excretion pattern; approximately 38.3%, 28%, 10.5%, and less than 1% was excreted in urine, in feces, as CO_2, and as volatile organics, respectively (Table J-3 and Table J-4).

The biliary excretion study conducted in rats following a single intraperitoneal injection of 50 mg/kg [^{14}C]-*o*-chloropyridine showed that approximately 27% of the dose was excreted in bile within 4 hours after dosing (Table J-5). Because only 8% of a 50 mg/kg intaperitoneal dose of [^{14}C]-*o*-chloropyridine was excreted in feces within 24 hours in a pilot study (data not shown), a significant recirculation of the dose from the lower gastrointestinal tract can be expected.

A profile of urine from oral gavage administration is shown in Figure J-1. At least nine peaks were detected of which several were not retained by reverse phase chromatography, but did not cleave by acid hydrolysis. Less than 1% of the recovered ^{14}C eluted at the retention time for the parent compound suggesting significant metabolism of *o*-chloropyridine following oral gavage administration. Based on the metabolism of other pyridines[63-65], the urinary excretion of several metabolites was anticipated. Metabolism may include C- and N-oxidation with some conjugation possible. N-Methylation is a significant route of metabolism of pyridine compounds in certain species, including the rat[66].

Urinary metabolite fractions were isolated using semipreparative HPLC for further characterization. Analyses of three major fractions by NMR spectroscopy and MS indicated disubstituted pyridine structures. Data from a proton NMR spectrum substantiated the structure for one metabolite to contain a mercapturic acid as one substituent and a hydroxyl group as the other substituent on the pyridine ring (Figure J-2). However, it was uncertain whether the hydroxyl group was located at the 4 or 5 position on the pyridine ring. Mass spectral and proton NMR data indicated the structures for two other metabolites to be consistent with hydroxylated *o*-chloropyridines. One of these was confirmed to be 2-chloro-5-hydroxypyridine by comparison with a synthetic standard (Figure J-2). For the other metabolite, the position of the hydroxyl substitution could not be confirmed (Figure J-2). The HPLC retention time and proton NMR spectra of the third metabolite did not match with those of a synthetic standard of 2-chloro-4-pyridinol (Figure J-2).

Profiling bile after a single intraperitoneal injection of 50 mg/kg [^{14}C]-*o*-chloropyridine showed that the excretion of metabolites in bile was less complex than in urine following oral exposure potentially due to route and/or matrix differences. Incubation of the chromatographically separated bile fractions with β-glucuronidase obtained from *E. coli* showed shifts in six of the eight metabolite fractions analyzed, indicating the presence of several glucuronide metabolites (results not shown).

J.3.2. Toxicokinetics

o-Chloropyridine radioactive equivalents in blood (ng-equivalents/g blood) over time in male rats given a single gavage dose of 10 mg [^{14}C]-*o*-chloropyridine/kg body weight are presented in Table J-6. The blood concentration versus time data and estimated toxicokinetic parameters are shown in Figure J-3. Absorption of *o*-chloropyridine following gavage administration was rapid with a rapid decrease in the concentration of radiolabel (*o*-chloropyridine equivalents) in blood. The data were best fit using a two-compartment model. The maximum concentration was reached within 30 minutes. The estimated distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half-lives, based on the total radioactivity, were 1.14 and 46 hours, respectively.

The blood concentrations of *o*-chloropyridine (ng/g blood) versus time in rats after a single intravenous injection of 1 mg/kg [^{14}C]-*o*-chloropyridine are shown in Table J-7 and Figure J-4. The maximum concentration of *o*-chloropyridine in blood, 567 ng/g, was measured at the earliest collection time (0.083 hours postdose). By 3 hours postdosing, the concentration of *o*-chloropyridine in the blood was too low to detect. A two-compartment model resulted in the best fits to the blood *o*-chloropyridine concentration-time data. (Data from one rat was poorly fitted by the simulation at the last two time points. For this reason, data for this rat was excluded from the calculation of the mean elimination half-life.) The distribution half-life ($t_{1/2\alpha}$) of *o*-chloropyridine in the blood was a very rapid 0.103 hours. The elimination half-life ($t_{1/2\beta}$) of *o*-chloropyridine in the blood was 1.04 hours (Figure J-4).

J.4. Summary

The objectives of these studies were to determine disposition and metabolism of *o*-chloropyridine in male rats after a single oral or injected (i.e., intravenous or intraperitoneal) dose and also to obtain basic toxicokinetic parameters following gavage and intravenous administration in male rats. Excretion and disposition studies were conducted in rats with oral

gavage doses of 0.1 and 10 mg/kg [^{14}C]-*o*-chloropyridine. Results were comparable between the two doses indicating no dose-related difference in disposition. Absorption of *o*-chloropyridine following gavage administration was rapid with the maximum blood concentration reached within 30 minutes. Approximately 82% of the radiolabeled dose was recovered within 72 hours in both studies: 36% to 39% of the dose was excreted in the urine, 28% in feces, 10% to 13% was eliminated as CO_2, and approximately 1% was excreted as volatile organics. The only tissues with appreciable tissue:blood ratios were the liver and the kidney. The estimated distribution ($t_{1/2\alpha}$) and elimination ($t_{1/2\beta}$) half-lives in blood, based on the total radioactivity, were 1.14 and 46 hours, respectively. Attempts were made to identify three urinary metabolites that comprised a large percentage of total radioactivity eluted in urine. Data from NMR spectroscopy and MS analyses indicated disubstituted pyridine structures. One of the metabolites contained mercapturic acid as one substituent and a hydroxyl group as the other, although it was uncertain as to whether the hydroxyl group was located at the 4 or 5 position on the pyridine ring. Data for the other two metabolites are consistent with hydroxylated *o*-chloropyridines, one of which was confirmed as 2-chloro-5-hydroxypyridine by comparison with a synthetic standard. Approximately 27% of a single intraperitoneal dose of

50 mg/kg was excreted in bile within 4 hours of dosing. Incubation of HPLC-fractionated bile with β-glucuronidase obtained from *E. coli* showed shifts in six of the eight metabolite fractions indicating the presence of several glucuronide metabolites.

Table J-1. Disposition of Radioactivity in Male F344 Rats 72 Hours Following a Single Oral Gavage Dose of 10 mg/kg [^{14}C]-*o*-Chloropyridine[a]

End of Collection Period (Hours)	Urine	Feces	Exhaled CO_2	Volatile Organics	Total
6	16.3 ± 3.8	–[b]	4.38 ± 0.61	0.954 ± 0.298	21.6 ± 4.0
12	26.0 ± 2.5	3.43 ± 4.7	9.36 ± 1.04	1.06 ± 0.31	39.8 ± 4.7
24	32.0 ± 1.8	25.0 ± 2.3	11.3 ± 1.2	1.11 ± 0.33	69.4 ± 2.6
48	34.8 ± 1.5	28.0 ± 2.6	12.1 ± 1.2	1.13 ± 0.33	76.0 ± 2.8
72	35.9 ± 1.6[c]	28.4 ± 2.5	12.5 ± 1.2	1.14 ± 0.33	78.0 ± 3.0
Disposition Summary					
Excreta					78.0 ± 3.0
Residual Carcass[d]					0.485 ± 0.093
Tissues[e]					3.43 ± 2.9
Total Dose Recovered					81.9 ± 2.9

[a]Data are presented as cumulative percentage of the dose (mean ± standard deviation) for five rats.
[b]No collection was scheduled for this time interval.
[c]Includes cage rinse.
[d]Percent dose recovered in the residual carcass less the percent dose measured in individual tissues: adipose, blood, muscle, and skin.
[e]Percent dose recovered in the following tissues: adipose, bladder, blood, brain, heart, kidney, liver, lung, muscle, skin, spleen, and testis. Also included were cecum, large and small intestine, and stomach, all with contents.

Table J-2. Tissue Distribution of Radioactivity in Male F344 Rats 72 Hours Following a Single Oral Gavage Dose of 10 mg/kg [^{14}C]-*o*-Chloropyridine[a]

Tissue	*o*-Chloropyridine Equivalents in Tissue (ng-Eq/g Tissue)	Tissue:Blood Ratio	Dose in Total Tissue (%)[b]
Adipose[c]	140 ± 84	0.417 ± 0.270	0.0910 ± 0.0545
Bladder	524 ± 57	1.53 ± 0.25	0.00185 ± 0.00038
Blood	346 ± 35	Unity	0.167 ± 0.018
Brain	101 ± 10	0.293 ± 0.033	0.00646 ± 0.00134
Cecum[d]	NA	NA	0.0826 ± 0.0087
Heart	364 ± 39	1.06 ± 0.16	0.0110 ± 0.0014
Intestine, Large[d]	NA	NA	0.0652 ± 0.0144
Intestine, Small[d]	NA	NA	0.106 ± 0.007
Kidney	2,500 ± 309	7.27 ± 0.94	0.176 ± 0.007
Liver	3,660 ± 471	10.6 ± 1.5	1.47 ± 0.15
Lung	442 ± 34	1.29 ± 0.15	0.0158 ± 0.0014
Muscle[c]	153 ± 18	0.445 ± 0.056	0.682 ± 0.083
Skin, Ear	291 ± 34	0.845 ± 0.110	0.459 ± 0.055
Spleen	506 ± 35	1.48 ± 0.19	0.0108 ± 0.0014
Stomach[d]	NA	NA	0.0636 ± 0.0072
Testis	139 ± 7	0.404 ± 0.039	0.0158 ± 0.0010
Carcass[e]	NA	NA	0.485 ± 0.093

NA = Not applicable.

[a]Data are presented as mean ± standard deviation for five rats.

[b]Percent dose was calculated using the following values for the mass of total tissue expressed as percent of body weight: adipose, 7.0%; blood, 5.2%; muscle, 48.0%; and skin, 17% (taken from Donaldson[67]; Adolph[68]; Supplee et al.[69]; Caster et al.[70]; Bischoff et al.[71]; and Lutz et al.[72]).

[c]Adipose and muscle values are averaged results for two sampling locations.

[d]Includes contents.

[e]Carcass value is based on the residual digested carcass after the removal of the listed tissues (i.e., percent dose measured in adipose, blood, muscle, and skin was subtracted from the total percent dose measured in the carcass).

Table J-3. Disposition of Radioactivity in Male F344 Rats 72 Hours Following a Single Oral Gavage Dose of 0.1 mg/kg [^{14}C]-*o*-Chloropyridine[a]

End of Collection Period (Hours)	Urine	Feces	Exhaled CO_2	Volatile Organics	Total
6	18.8 ± 3.0	–[b]	4.93 ± 0.63	0.518 ± 0.058	24.3 ± 3.1
12	29.1 ± 0.9	3.06 ± 2.30	8.47 ± 0.84	0.564 ± 0.084	41.2 ± 3.3
24	34.8 ± 0.7	24.0 ± 3.5	9.53 ± 0.83	0.579 ± 0.089	68.9 ± 3.4
48	37.0 ± 0.8	27.2 ± 3.0	10.2 ± 0.9	0.587 ± 0.088	75.0 ± 3.1
72	38.3 ± 0.8[c]	28.0 ± 2.5	10.5 ± 0.9	0.595 ± 0.089	77.4 ± 2.6
Disposition Summary					
Excreta					77.4 ± 2.6
Residual Carcass[d]					0.624 ± 0.176
Tissues[e]					3.88 ± 0.24
Total Dose Recovered					81.9 ± 2.6

[a]Data are presented as cumulative percentage of the dose (mean ± standard deviation) for five rats.
[b]No collection was scheduled for this time interval.
[c]Includes cage rinse.
[d]Percent dose recovered in the residual carcass less the percent dose measured as individual tissues: adipose, blood, muscle, and skin.
[e]Percent dose recovered in the following tissues: adipose, bladder, blood, brain, heart, kidney, liver, lung, muscle, skin, spleen, and testis. Also included were cecum, large and small intestine, and stomach, all with contents.

Table J-4. Tissue Distribution of Radioactivity in Male F344 Rats 72 Hours Following a Single Oral Gavage Dose of 0.1 mg/kg [^{14}C]-*o*-Chloropyridine[a]

Tissue	*o*-Chloropyridine Equivalents in Tissue (ng-Eq/g Tissue)	Tissue:Blood Ratio	Dose in Total Tissue (%)[b]
Adipose[c]	1.43 ± 0.44	0.382 ± 0.122	0.0867 ± 0.0253
Bladder	7.69 ± 1.46	7.69 ± 1.46	0.00221 ± 0.00055
Blood	3.76 ± 0.07	Unity	0.171 ± 0.007
Brain	1.28 ± 0.07	0.341 ± 0.018	0.00854 ± 0.00076
Cecum[d]	NA	NA	0.153 ± 0.040
Heart	4.69 ± 0.23	1.25 ± 0.069	0.0147 ± 0.0010
Intestine, Large[d]	NA	NA	0.0416 ± 0.0095
Intestine, Small[d]	NA	NA	0.125 ± 0.017
Kidney	27.1 ± 3.03	7.22 ± 0.93	0.185 ± 0.013
Liver	49.0 ± 4.8	13.0 ± 1.27	1.44 ± 0.06
Lung	5.68 ± 0.276	1.51 ± 0.086	0.0214 ± 0.0034
Muscle[c]	2.58 ± 0.37	0.687 ± 0.109	1.08 ± 0.15
Skin, Ear	3.16 ± 0.40	0.839 ± 0.105	0.468 ± 0.066
Spleen	6.33 ± 0.33	1.68 ± 0.10	0.0118 ± 0.0001
Stomach[d]	NA	NA	0.0578 ± 0.0157
Testis	1.64 ± 0.06	0.437 ± 0.015	0.0172 ± 0.0010
Carcass[e]	NA	NA	2.42 ± 0.07

NA = Not applicable.
[a]Data are presented as mean ± standard deviation for five rats.
[b]Percent dose was calculated using the following values for the mass of total tissue expressed as percent of body weight: adipose, 7.0%; blood, 5.2%; muscle, 48.0%; and skin, 17% (taken from Donaldson[67]; Adolph[68]; Supplee et al.[69]; Caster et al.[70]; Bischoff et al.[71]; and Lutz et al.[72]).
[c]Adipose and muscle values are averaged results for two sampling locations.
[d]Includes contents.
[e]Carcass value is based on the residual digested carcass after the removal of the listed tissues (i.e., percent dose measured in adipose, blood, muscle, and skin was subtracted from the total percent dose measured in the carcass).

Table J-5. Excretion of Radioactivity in Bile in Male F344 Rats Following a Single Intraperitoneal Injection of 50 mg/kg [^{14}C]-*o*-Chloropyridine[a]

End of Collection Period (hours)	Bile
0.5	1.99 ± 0.69
1.0	6.10 ± 2.59
1.5	9.86 ± 4.55
2.0	13.5 ± 5.7
2.5	16.6 ± 6.3
3.0	20.8 ± 7.9
3.5	23.6 ± 8.2
4.0	26.9 ± 8.7

[a]Data are presented as cumulative percentage of the dose (mean ± standard deviation) for five rats.

Table J-6. Total Radioactive Equivalents in Blood Over Time in Male F344 Rats Following a Single Oral Gavage Dose of 10 mg/kg [^{14}C]-*o*-Chloropyridine[a]

Time After Dosing (hours)	*o*-Chloropyridine Equivalents in Blood (ng-Eq/g Blood)	Dose in Total Blood (%)
0.083	2,062 ± 316	1.07 ± 0.16
0.25	3,376 ± 738	1.75 ± 0.38
0.50	3,807 ± 462	1.97 ± 0.24
1	3,278 ± 570	1.70 ± 0.29
1.5	2,687 ± 243	1.39 ± 0.12
2	2,192 ± 305	1.14 ± 0.16
3	1,573 ± 166	0.814 ± 0.086
4	1,224 ± 111	0.634 ± 0.057
5	1,153 ± 78	0.597 ± 0.040
6	1,092 ± 73	0.565 ± 0.038
12	917 ± 102	0.475 ± 0.053
24	724 ± 87	0.375 ± 0.045
48[b]	475 ± 58	0.246 ± 0.030
72[c]	353 ± 23	0.183 ± 0.012

[a]Data are presented as mean ± standard deviation for six rats.

Table J-7. Concentration of *o*-Chloropyridine and Total Radioactive Equivalents in Blood Over Time in Male F344 Rats Following a Single Intravenous Injectionof 1 mg/kg [^{14}C]-*o*-Chloropyridine[a]

Time After Dosing (Hours)	*o*-Chloropyridine (ng/g Blood)	Radiolabel *o*-Chloropyridine Equivalents (ng-Eq/g Blood)	Radiolabel Dose in Total Blood (%)
0.083	567 ± 23	632 ± 146	4.09 ± 0.99
0.17	437 ± 31	422 ± 39	2.76 ± 0.27
0.25	360 ± 31	356 ± 5	2.30 ± 0.06
0.50	220 ± 20	286 ± 35	1.85 ± 0.26
1	123 ± 10	209 ± 37	1.35 ± 0.24
1.5	100 ± 43	138 ± 11	0.893 ± 0.075
2	73.2 ± 53.4	100 ± 9	0.646 ± 0.069
3	ND	74.9 ± 6.1	0.484 ± 0.041
4	ND	74.9 ± 4.8	0.483 ± 0.025

ND = Not determined.
[a]Data are presented as mean ± standard deviation for four rats.

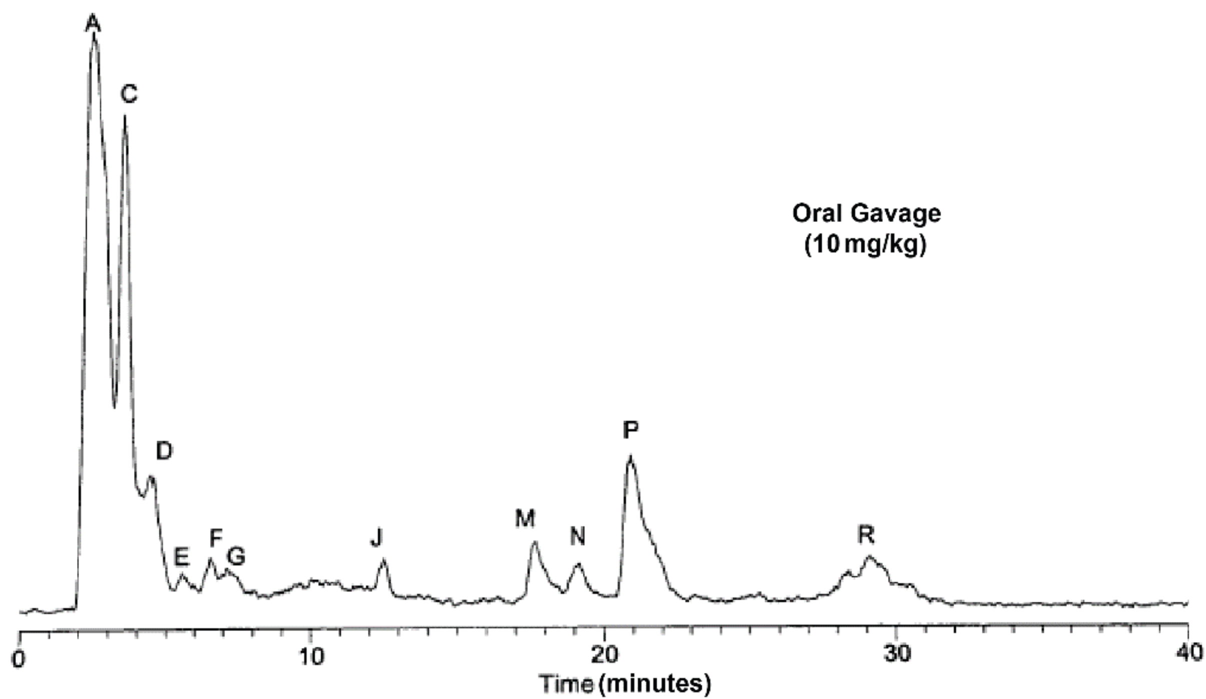
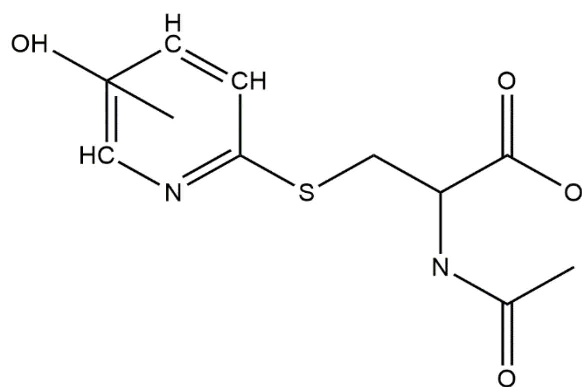
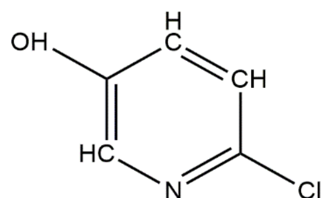


Figure J-1. [^{14}C]-Labeled Urinary Metabolites in Male F344 Rats Following a Single Oral Gavage Dose of 10 mg/kg [^{14}C]-*o*-Chloropyridine

Metabolites were determined in a composite (0 to 24 hours) urine sample from one rat.





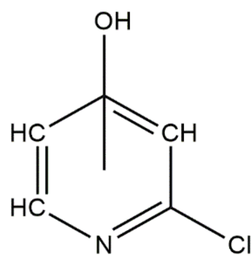
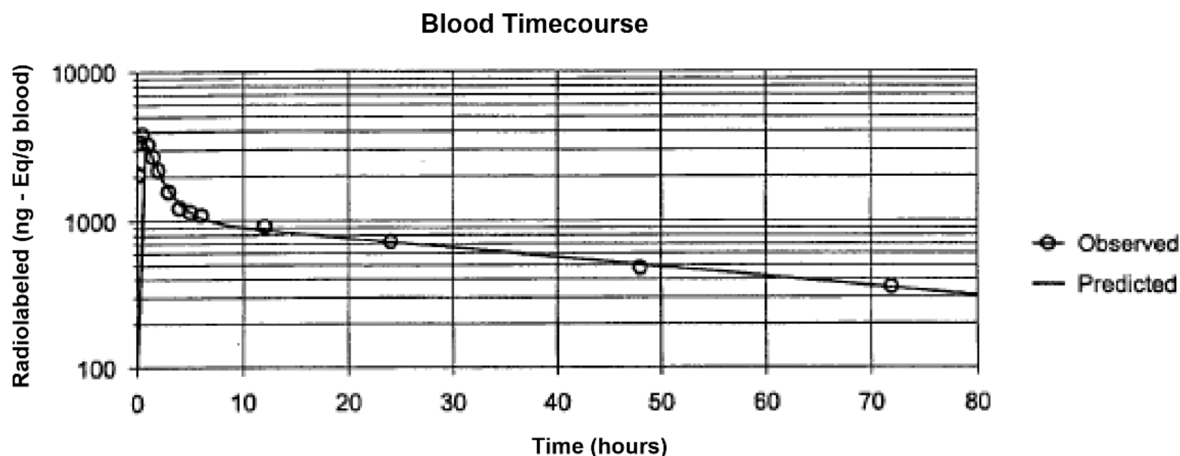


Figure J-2. Disubstituted Urinary Metabolites of *o*-Chloropyridine in Male F344 Rats



Toxicokinetic Parameters

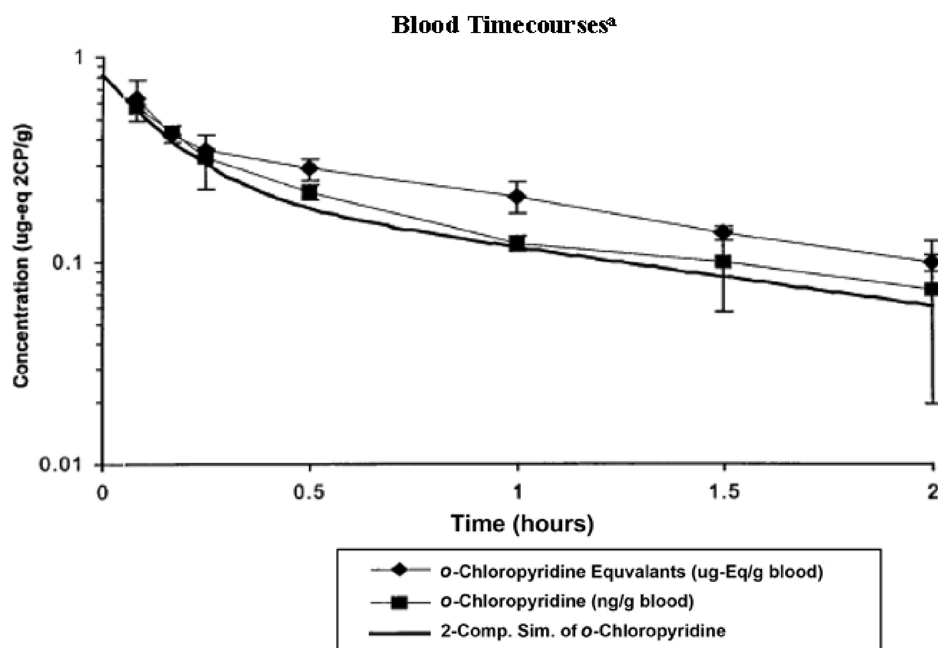
Parameter	Estimate	Std Error	CV%	UnivarCI Lower	UnivarCI Upper
A	2129	110.1	5.17	1880	2378
B	546.5	51.16	9.36	430.7	662.2
K01	6.71	0.540	8.05	5.48	7.93
Alpha	0.607	0.0613	10.1	0.468	0.746
Beta	0.0151	0.00354	23.5	0.00706	0.0231

Distribution of half-life of radiolabel: $t_{1/2}$ **= 0.693/alpha = 1.14 hours**
Elimination of half-life of radiolabel: $t_{1/2}$ **= 0.693/beta = 46.0 hours**

Figure J-3. Blood Timecourse and Toxicokinetic Parameter Estimates of *o*-Chloropyridine in Male F344 Rats Following a Single Oral Gavage Dose of 10 mg/kg [^{14}C]-*o*-Chloropyridine

Data were fitted using a two-compartment model. Constants A and B are intercepts on the y axis for each exponential segment of the curve and β and α are the elimination and distribution rate constants, respectively.

Blood Timecourses[a]



Toxicokinetic Parameters

Values from 2-Compartment Simulation of *o*-Chloropyridine in Blood[b]

Parameter	Mean	Standard Deviation	CV%
Volume of distribution (mL/kg)	982	221	22.5
Alpha (hour-1)	6.71	3.42	50.9
Beta (hour-1)	0.669	0.028	4.22
Distribution half-life (hours)	0.103	NA	NA
Elimination half-life (hours)	1.04	NA	NA
Clearance (mL/min/kg)	29.8	2.5	8.39

NA=Not applicable. Samples beyond 2 hours contained insufficient radioactivity for analysis.
[a]Values are mean ± standard deviation for four rats.
[b]Values are mean ± standard deviation for three rats; rat number 5 was dropped from the simulation due to variability in the concentration of *o*-chloropyridine present at the latest two time points for this animal; the simulation was skewed if rat number 5 was included.

Figure J-4. Blood Timecourse and Toxicokinetic Parameter Estimates for *o*-Chloropyridine and Radiolabel (*o*-Chloropyridine Equivalents) in Blood of Male F344 Rats Following a Single Intravenous Injection of 1 mg/kg [^{14}C]-*o*-Chloropyridine

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