National Toxicology Program
Toxicity Report Series
Number 72

NTP Technical Report on the Toxicity Studies of

Sodium Dichromate Dihydrate

(CAS No. 7789-12-0)

Administered in Drinking Water to Male and Female F344/N Rats and B6C3F$_1$ Mice and Male BALB/c and *am3*-C57BL/6 Mice

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FOREWORD

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John R. Bucher, Ph.D., Study Scientist

National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication No. 07-5964

National Institutes of Health
Public Health Service
U.S. Department of Health and Human Services

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

K.M. Abdo, Ph.D., Study Scientist
J.R. Bucher, Ph.D., Study Scientist
R.A. Herbert, D.V.M., Ph.D, Study Pathologist
R.S. Chhabra, Ph.D.
M.J. Hooth, Ph.D.
G.E. Kissling, Ph.D.
A.P. King-Herbert, D.V.M.
D.E. Malarkey, D.V.M., Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
M.K. Vallant, B.S., M.T.
K.L. Witt, M.S.

Southern Research Institute

Conducted studies in F344/N rats and B6C3F$_1$ mice (Study 1) and evaluated pathology findings

C.D. Hébert, Ph.D., Principal Investigator
J.E. Heath, D.V.M.
D. Hoskins
R.B. Thompson, D.V.M., Ph.D.

Battelle Columbus Operations

Conducted studies in male B6C3F$_1$, BALB/c, and am3-C57BL/6 mice (Study 2) and evaluated pathology findings

M.R. Hejtmancik, Ph.D., Principal Investigator
L.M. Fomby, D.V.M., Ph.D.
T.A. Peace, D.V.M.
D.M. Sells, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
K.J. Cimon, D.V.M, M.S.
J.C. Peckham, D.V.M., Ph.D.
C.C. Shackelford, D.V.M., M.S., Ph.D.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides and prepared pathology report for F344/N rats and B6C3F$_1$ mice (Study 1) (March 10, 2003)

A. Suttie, B.V.Sc., Ph.D., Chairperson
ILS, Inc.
K.J. Cimon, D.V.M, M.S.
Experimental Pathology Laboratories, Inc.
M.R. Elwell, D.V.M., Ph.D.
Pfizer
J.R. Hailey, D.V.M.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
A. Nyska, D.V.M.
National Toxicology Program
J.C. Peckham, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
C.C. Shackelford, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
H. Shive, B.S., Observer
North Carolina State University
Y. Tani, D.V.M., Ph.D., Observer
National Toxicology Program

NTP Pathology Working Group

Evaluated slides and prepared pathology report for male B6C3F$_1$, BALB/c, and am3-C57BL/6 mice (Study 2) (April 1, 2004)

A. Suttie, B.V.Sc., Ph.D., Chairperson
ILS, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program

Constella Group, Inc.

Provided statistical analyses

P.W. Crockett, Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.
J.T. Scott, M.S.

Biotechnical Services, Inc.

Prepared Toxicity Study Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
J.I. Powers, M.A.P.
D.C. Serbus, Ph.D.

PEER REVIEW

The draft report on the toxicity and comparative toxicity studies of sodium dichromate dihydrate was evaluated by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that this Toxicity Study Report presents the experimental results and conclusions fully and clearly.

Gary P. Carlson, Ph.D.
School of Health Sciences
Purdue University
West Lafayette, IN

Nancy I. Kerkvliet, Ph.D.
Department of Environmental and Molecular Toxicology
Oregon State University
Corvallis, OR

Richard M. Sedman, Ph.D.
California Office of Environmental Health Hazard Assessment
Oakland, CA

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SUMMARY

Background
Sodium dichromate dihydrate is used for electroplating, leather tanning, and textile manufacturing. As a result, sodium dichromate dihydrate and other chromium compounds have been found in drinking water supplies. The NTP conducted a series of tests to determine the effects of sodium dichromate dihydrate on rodents. This report examines short-term (3-month) toxicology tests in rats and three strains of mice. These studies were performed to determine doses and the most appropriate test species for future long-term studies of sodium dichromate dihydrate.

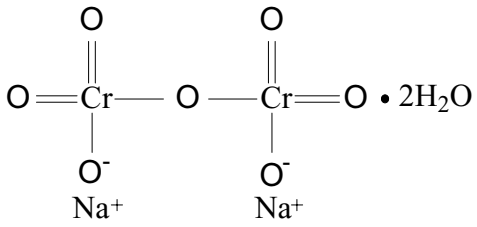
Methods
We gave rats and mice sodium dichromate dihydrate dissolved in drinking water for three months. In one study, groups of ten male and ten female rats and mice received concentrations of 62.5, 125, 250, 500, or 1,000 milligrams (mg) of sodium dichromate dihydrate per liter (L) of water. In a second study, groups of five male mice of three different strains received concentrations of 6.25, 125, or 250 mg/L for three months. Similarly sized groups for each sex and species were given plain water and served as the controls. Tissues from 35 sites were examined for each animal in the first study. In the second study, tissues from the liver and stomach of each animal were examined microscopically.

Results
All animals survived to the end of the studies. In the first study, rats receiving 500 mg/L or more and mice receiving 125 mg/L or more weighed less than the controls. Sodium dichromate dihydrate caused a form of anemia in both rats and mice. Male and female rats exposed to 1,000 mg/L had ulceration, hyperplasia, and metaplasia of the forestomach and histiocytic infiltration of the small intestine. Mice exposed to sodium dichromate dihydrate had hyperplasia of the small intestine.

In the second study, mice also had hyperplasia of the small intestine.

Conclusions
Exposure to sodium dichromate dihydrate caused hyperplasia and ulceration of the stomach in rats and an anemia and lesions of the small intestine in rats and mice.

ABSTRACT



Sodium Dichromate Dihydrate

CAS No. 7789-12-0

Chemical Formula: $Na_2Cr_2O_7 \cdot 2H_2O$ Molecular Weight: 298.0

Synonyms: Chromic acid; dichromic acid; disodium salt, dihydrate; disodium dichromate dihydrate; chromium VI

Sodium dichromate dihydrate is one of a number of inorganic compounds containing hexavalent chromium (CR VI) found in drinking water supplies as a contaminant resulting from various industrial processes including electroplating operations, leather tanning, and textile manufacturing. Because of the lack of adequate experimental data on the toxicity and carcinogenicity of hexavalent chromium ingested orally, and because hexavalent chromium has been found in human drinking water supplies, the California Congressional delegation and the California Environmental Protection Agency nominated hexavalent chromium to the NTP for study. In study 1, male and female F344/N rats and B6C3F$_1$ mice were exposed to sodium dichromate dihydrate (greater than 99% pure) in drinking water for 3 months. In study 2, sodium dichromate dihydrate was administered in drinking water to male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice for 3 months. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, and mouse peripheral blood erythrocytes.

In study 1, groups of 10 male and 10 female F344/N rats and B6C3F$_1$ mice were given drinking water containing 0, 62.5, 125, 250, 500, or 1,000 mg sodium dichromate dihydrate/L for 3 months (equivalent to average daily doses of approximately 5, 10, 17, 32, or 60 mg sodium dichromate dihydrate/kg body weight to rats and 9, 15, 26, 45, or 80 mg/kg to mice). On a molecular weight basis, these doses are equivalent to approximately 1.7, 3.5, 5.9, 11.2, and 20.9 mg hexavalent chromium/kg body weight per day to rats and 3.1, 5.2, 9.1, 15.7, and 27.9 mg/kg per day to mice.

Additional groups of 10 rats per sex were exposed to the same concentrations of sodium dichromate dihydrate for 4 weeks. All rats and mice survived to the end of the study. Reduced body weights occurred in 500 and 1,000 mg/L male rats, 1,000 mg/L female rats, and in male and female mice exposed to 125 mg/L or greater. Water consumption by male and female rats exposed to 250 mg/L or greater and male and female mice exposed to 125 mg/L or greater was generally less than that by the control groups, and decreases in urine volume and increases in urine specific gravity in rats were related to reduced water consumption. Exposure to sodium dichromate dihydrate caused a microcytic hypochromic anemia in rats and mice, but the severity was less in mice. Serum cholesterol and triglyceride concentrations were decreased in rats. Increased bile acid concentrations in exposed groups of rats may have been due to altered hepatic function.

The incidences of histiocytic cellular infiltration were generally significantly increased in the duodenum of rats and mice, the liver of female rats, and the mesenteric lymph node of mice exposed to 125 mg/L or greater. Significantly increased nonneoplastic lesions (focal ulceration, regenerative epithelial hyperplasia, and squamous epithelial metaplasia) occurred in the glandular stomach of male and female rats exposed to 1,000 mg/L. Incidences of epithelial hyperplasia of the duodenum were significantly increased in all exposed groups of mice.

In study 2, sodium dichromate dihydrate was administered in drinking water to groups of 10 male B6C3F$_1$, 10 male BALB/c, and five male *am3*-C57BL/6 mice for 3 months at exposure concentrations of 0, 62.5, 125, or 250 mg/L (equivalent to average daily doses of approximately 8, 15, or 25 mg/kg sodium dichromate dihydrate or 2.8, 5.2, or 8.7 mg/kg chromium to B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice). All mice in study 2 survived until study termination. Mean body weights of 125 and 250 mg/L B6C3F$_1$ and BALB/c mice and all exposed groups of *am3*-C57BL/6 mice were less than those of the control groups. Mice exposed to 250 mg/L consumed less water than the control groups. Exposure concentration-related decreases in mean red cell volumes and mean red cell hemoglobin values were observed in all three mouse strains. Erythrocyte counts were increased in exposed B6C3F$_1$ and BALB/c mice but not in *am3*-C57BL/6 mice. Changes in organ weights were generally consistent with reduced body weights in exposed groups in all mouse strains. No biologically significant differences in reproductive parameters were observed in any strain.

Histiocytic cellular infiltration and epithelial hyperplasia of the duodenum occurred in most mice exposed to 125 or 250 mg/L, and the incidences of these lesions were increased in the 62.5 mg/L group compared to controls. Secretory depletion was present in the pancreas of most mice exposed to 125 or 250 mg/L. The incidences of glycogen depletion of the liver were significantly increased in male B6C3F$_1$ mice exposed to 125 or 250 mg/L and in all exposed groups of male *am3*-C57BL/6 mice. The incidence of histiocytic cellular infiltration in the mesenteric lymph node was significantly increased in the 250 mg/L group of male *am3*-C57BL/6 mice.

Sodium dichromate dihydrate was mutagenic in *S. typhimurium* strains TA100 and TA98 and in *E. coli* strain WP2 uvrA pKM101 with and without induced rat liver S9 enzymes. The results of four micronucleus tests conducted in the three strains of mice from studies 1 and 2 were mixed. In study 1, no significant increases were seen in micronucleated normochromatic erythrocytes in peripheral blood samples from male or female B6C3F_1 mice; there was a decrease in the percentage of polychromatic erythrocytes among total erythrocytes (an indication of bone marrow toxicity), but the changes were small and not well correlated with exposure concentrations. In study 2, a significant exposure concentration-related increase ($P<0.001$) in micronucleated normochromatic erythrocytes was seen in *am3*-C57BL/6 male mice. An equivocal increase in micronucleated erythrocytes was noted in male B6C3F_1 mice, based on a small increase in micronucleated normochromatic erythrocytes that did not reach statistical significance. No increase in micronucleated normochromatic erythrocytes was observed in male BALB/c mice. No significant effect of sodium dichromate dihydrate exposure on the percentage of polychromatic erythrocytes was observed in any of the three micronucleus tests conducted in study 2.

In summary, administration of sodium dichromate dihydrate in the drinking water to F344/N rats and B6C3F_1 mice resulted in focal ulceration, hyperplasia, and metaplasia in the glandular stomach at the limiting ridge in rats in the 1,000 mg/L group and evidence of increased histiocytic infiltration in the liver (female), duodenum of the small intestine, and/or pancreatic lymph nodes at concentrations as low as 62.5 mg/L, the lowest concentration studied. In addition, a microcytic, hypochromic anemia occurred at all exposure concentrations and was considered evidence of a toxic response resulting from absorption of Cr VI following oral ingestion in rats. A similar, but less severe, anemia was evident in mice receiving drinking water containing sodium dichromate dihydrate; histiocytic infiltration was noted in the duodenum of all three strains studied (B6C3F_1, BALB/c, and *am3*-C57BL/6) at all concentrations employed, in the mesenteric lymph nodes at 125 mg/L or greater in the B6C3F_1 strain, and at 250 mg/L in the *am3*-C57BL/6 strain. There was no consistent evidence of hepatocyte injury in mice in any of the strains tested. Variations in glycogen content were considered more likely related to diminished food intake than to the toxicity of sodium dichromate dihydrate.

INTRODUCTION

CHEMICAL AND PHYSICAL PROPERTIES

Sodium dichromate dihydrate (CAS No. 7789-12-0) exists in solid form as reddish to bright orange crystals, which become anhydrous when heated to approximately 100° C. The anhydrous form has a melting point of 356.7° C (*Merck*, 1996). The water solubility of sodium dichromate dihydrate is 230 g/L at 0° C. Sodium dichromate dihydrate is one of a number of inorganic compounds that contain chromium in the hexavalent state (Cr VI). Other representatives of this class of compounds include sodium, potassium, calcium, ammonium chromate, potassium dichromate, and chromic acid. The physical properties and water solubility of these compounds vary considerably.

Chromium is a group VI transition metal and occurs in the earth's crustal rock at a concentration averaging 122 ppm. It has six oxidation states. The most stable states are the metallic chromium (Cr), trivalent chromium (Cr III), and hexavalent chromium (Cr VI). Cr VI is typically present in complexes with halide (chromyl chloride) and oxygen ligands (chromium trioxide, chromate, dichromate). Cr VI is easily reduced to Cr III in acidic solutions containing organic molecules such as proteins, DNA, or glutathione. Glutathione is also capable of reducing Cr VI at neutral pH at a slower rate than under acidic conditions (Zhitkovich, 2005).

PRODUCTION, USE, AND HUMAN EXPOSURE

Metallic chromium is produced by reduction of chromite ore. Sodium dichromate is produced by roasting chromite ore with soda ash and is used for the production of other chromium compounds (Hartford, 1979; Westbrook, 1979). Metallic chromium is used in the metallurgical industry for the production of stainless steel and ferrous and nonferrous alloys. The major uses of chromium in the chemical and manufacturing industries include the production of chromium pigments and in metal finishing, leather tanning, and wood preservation (Barnhart, 1997). Chromium enters the environment from combustion processes and ore processing mainly as chromium (III) oxide. Cr VI has been detected in fly ash from power plants (Stern, 1982). Both Cr III and Cr VI enter water resources by leaching from soil or from industrial contamination (Pellerin and Booker, 2000) as well as from fallout from the atmosphere. These authors also reported that a hexavalent chromium (Cr VI) concentration as high as 580 µg/L was found in a groundwater monitoring well in Hinkley, California. Detectable levels of hexavalent chromium have been reported in approximately 30% of the drinking water sources monitored in California, which uses a 1 µg/L detection limit for purposes of reporting (CDHS, 2004). Exposure of the public to chromium occurs through food, water, and air. The highest exposure to Cr VI occurs occupationally to workers involved in chrome plating, chromate production, and

stainless steel welding. Exposure in these situations is typically by inhalation or dermal contact. Additional information on human exposure to chromium and chromium compounds can be found in the Toxicological Profile for Chromium (ATSDR, 2000).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Chromium and chromium compounds are absorbed after oral, dermal, or inhalation exposure (Wahlberg and Skog, 1965; Wahlberg, 1970; Kerger *et al*., 1997; Mancuso, 1997). Most studies of absorption of Cr III or Cr VI after oral administration to rodents find that only 1% or 2% of the administered dose is bioavailable, whereas similar studies with humans report somewhat higher numbers (ATSDR, 2000), particularly for Cr VI. It is thought that Cr VI is poorly absorbed when ingested due to its rapid reduction to the less soluble Cr III in the presence of food and the acidic environment encountered in the stomach (Sutherland *et al*., 2000). Cr III is less efficiently absorbed than Cr VI compounds, and this is attributed to a difference in their respective methods of transport into cells. Cr VI enters cells by facilitated diffusion via nonspecific anion channels, while Cr III enters cells by passive diffusion or phagocytosis of precipitates resulting in much lower uptake (ATSDR, 2000). However, one study in rats (Sayato *et al*., 1980) showed absorption of either valence state was similar after oral administration.

Human and animal studies showed that chromium is widely distributed in the body after exposure to Cr VI, with liver, kidney, spleen, and bone having higher concentrations than other tissues (Coogan *et al*., 1991; Kargacin *et al*., 1993; Mancuso, 1997). Rats consuming up to 10 ppm Cr VI as potassium chromate in their drinking water for 44 weeks had elevated chromium levels in bone, kidney, liver (females only), and testis (males only) (Sutherland *et al.*, 2000). Other studies showed that chromium can cross the placenta (Casey and Hambidge, 1984; Saxena *et al*., 1990). Higher tissue chromium levels were found in rats receiving Cr VI orally than in those receiving an equivalent dose of Cr III (Costa, 1997). Following uptake by tissues, Cr VI is thought to undergo reduction to Cr III intracellularly, primarily by ascorbate, permitting the formation of stable coordination complexes of Cr III with a wide variety of cellular constituents (Sehlmeyer *et al*., 1990). Complexes of Cr III with DNA have been demonstrated and may result in mutagenicity and chromosomal damage (Zhitkovich, 2005).

Ingested Cr VI is excreted primarily in the feces because of its poor absorption. Absorbed chromium appears to be primarily excreted in the urine (Donaldson and Barreras, 1966; Sayato *et al*., 1980). O'Flaherty (1993, 1996) has constructed a physiologically-based pharmacokinetic model for Cr VI compounds in the rat, and the ATSDR (2000) has outlined distribution and clearance models for Cr III in humans.

Prior to the selection of rats and mice as the test species for the studies in this report, NTP conducted a comparative absorption study of sodium dichromate dihydrate (Cr VI) administered in the drinking water to F344/N rats,

$B6C3F_1$ mice, and Hartley guinea pigs. Concentrations of total chromium were determined in blood and kidney (Appendix G). Guinea pigs were chosen for study, in addition to rats and mice, because they more closely resemble humans in that they do not have a forestomach and they require vitamin C (a reducing agent) in their diet.

In all three species, chromium in blood and kidney increased with exposure concentration. Although differences were seen in the absolute amounts of chromium in kidney and blood, uptake as a function of exposure concentration did not appear to differ qualitatively in guinea pigs when compared to rats and mice. This suggests that the presence of a forestomach did not fundamentally alter the Cr VI handling in the gastrointestinal tract. Kidney chromium concentration was highest in rats, followed by mice and guinea pigs. For blood, guinea pigs had the highest concentrations followed by mice and rats. The concentrations of chromium in blood and kidney were in general agreement with values observed by Sutherland *et al*. (2000).

While differences were seen in the patterns of tissue chromium accumulation among the three species, the results for chromium concentrations in blood and kidney in rats and mice were in general agreement with expectations based on values reported in the literature. The chromium concentrations in the blood of the guinea pigs suggested somewhat greater absorption than did the concentrations in the blood of the rats or mice.

TOXICITY

Cr III is an essential trace element. The recommended daily intake of Cr III for adults ranges between 25 and 45 µg (IOM, 2001). Cr III deficiency contributes to glucose intolerance and diabetes mellitus (Type 2). Chromium appears to increase sensitivity to insulin. The mechanism involves increased insulin binding through increasing the number of insulin receptors and increasing insulin receptor phosphorylation in the presence of insulin and a low molecular weight chromium complex (Anderson, 1998).

Cr III is considered relatively nontoxic when ingested. Rats exposed to Cr III as chromium oxide (2,040 mg/kg per day) in the diet 5 days per week for 2 years (Ivankovic and Preussmann, 1975), rats exposed to Cr III as chromium trichloride (2.7 mg/kg per day) in drinking water for 1 year (MacKenzie *et al.*, 1958), rats exposed to Cr III as chromium chloride (9 mg/kg per day) or chromium picolinate (9 mg/kg per day) in the diet for 20 weeks (Anderson *et al.*, 1997), or rats exposed to Cr III as chromium acetate (0.46 mg/kg per day) in the drinking water for 2 to 3 years did not show signs of toxicity or adverse systemic effects. By contrast, ingestion of hexavalent chromium caused gastric lesions in rats (Samitz, 1970). Reports from one laboratory have indicated that hepato- and nephrotoxicity as well as hematological changes were seen in an unspecified strain of rats receiving 13.5 or 50 mg/kg potassium chromate by gavage (Kumar *et al*., 1985; Kumar and Barthwal, 1991; ATSDR, 2000). Rats receiving Cr VI as potassium chromate (13.5 mg/kg per day for 20 days) had increased hepatic lipid accumulation and changes

in the liver distribution of histochemically determined activities of alkaline phosphatase, glucose-6-phosphatase, cholinesterase, and lipase (Kumar *et al*., 1985). Hepatocyte cytoplasmic vacuolization was reported in male and female mice administered potassium dichromate (Cr VI) (equivalent to 1.1 to 32 mg/kg per day for males and 1.8 to 48 mg/kg per day for females) in feed for 9 weeks (NTP, 1996a). Other studies using comparable doses of various Cr VI compounds have failed to demonstrate hepatotoxic effects (ATSDR, 2000).

Renal effects observed in rats administered Cr VI (as potassium chromate, 13.5 mg/kg per day for 20 days) by gavage included increased accumulation of lipid, triglycerides, and phospholipids in different regions of the kidney than controls and inhibited kidney membrane enzymes (alkaline phosphatase, acid phosphatase, glucose-6-phosphatase, and lipase) (Kumar and Rana, 1982, 1984). Oliguria and proteinuria were observed in rats exposed to Cr VI (as sodium chromate, 100 mg/kg per day for 28 days) in drinking water (Diaz-Mayans *et al.*, 1986).

Rats and mice administered potassium dichromate by diet (8.4 and 9.8 mg Cr VI/kg per day for male and female rats, respectively; 32.2 and 48 mg Cr VI/kg for male and female mice, respectively) showed reduced mean red cell volume and mean red cell hemoglobin (NTP 1996a,b). This effect was not seen at lower exposure concentrations. Similar decreases were seen in a multigeneration study of mice given potassium dichromate in the diet. The decreases were seen in BALB/c males receiving Cr VI at 16 mg/kg per day and 36.7 mg/kg per day and BALB/c females receiving Cr VI at 7.8 mg/kg per day (NTP, 1997). Toxicity of Cr VI was not confirmed in all animal studies. In a drinking water study, rats given Cr VI (as potassium chromate, 3.6 mg/kg per day) for 1 year showed no evidence of hepato- or nephrotoxicity and no alteration in hematology or clinical chemistry parameters (MacKenzie *et al.*, 1958).

The health effects of ingested Cr VI are not well understood because saliva and gastric juices partly reduce Cr VI to the less absorbed and less toxic Cr III (IARC, 1990; De Flora *et al.*, 1997). Accordingly, it is anticipated that ingested Cr VI may produce toxic effects at the point of contact. Recent studies showed that the reduction process of Cr VI to Cr III might result in oxidative DNA damage (Costa, 1997; Singh *et al.*, 1998).

CARCINOGENICITY

A retrospective mortality study of a population living near a chromium smelting plant in the People's Republic of China found increased incidences of lung and stomach tumors as compared to those of the general population (Zhang and Li, 1987). The authors did not estimate exposure levels, nor was the size of the population specified. Other studies of chromate-exposed populations in Mexico, Scotland, Japan, and Southern California have failed to provide what the authors considered definitive evidence of an increase in cancer incidence or mortality rates (Proctor *et al.*, 2002).

In contrast to the results of human epidemiology studies with chromate exposure primarily through water, exposure to Cr VI compounds by inhalation has long been recognized as carcinogenic to humans. The United States Department of Health and Human Services, the United States Environmental Protection Agency, and the International Agency for Research on Cancer classified Cr VI compounds as human carcinogens based on increased incidences of lung cancers in workers in the chromium industry and in experimental animals exposed to these compounds by inhalation (IARC, 1990; Cohen *et al.,* 1993; NTP, 1998). The compounds that are listed as carcinogens included calcium chromate, chromium trioxide, lead chromate, strontium chromate, and zinc chromate.

Although Cr VI compounds are demonstrated carcinogens by the inhalation route, it has been suggested that the reductive capacity of the gut would be adequate to prevent Cr VI from being a carcinogen when ingested (De Flora *et al*., 1997; Proctor *et al.*, 2002). No adequate animal carcinogenicity study of Cr VI administered by ingestion was found in the literature. In one lifetime multigeneration drinking water study, potassium chromate at 9 mg Cr VI/kg body weight per day was not considered carcinogenic in mice (Borneff *et al.*, 1968). Although the incidence of forestomach tumors (papillomas and carcinomas) in mice receiving the chemical did not differ statistically from that of the controls, carcinomas of the forestomach were seen only in the mice receiving the chemical. The study is considered inadequate because the majority of the animals died early in the study (8 to 11 months) due to an ectromelia epidemic.

No evidence of carcinogenicity was observed in male or female rats fed diets containing chromium oxide at 2,040 mg Cr III/kg body weight per day, 5 days per week for 2 years. Moreover, no evidence of carcinogenicity was found in the offspring of these rats after 600 days of observation (Ivankovic and Preussmann, 1975).

GENETIC TOXICITY

Sodium dichromate dihydrate (Cr VI) is genotoxic in a number of *in vitro* and *in vivo* test systems, although responses are somewhat variable depending on protocol details and the type of chromium salt that is assayed. Thus, there are some conflicting results in the literature, but overall, the data clearly indicate that in appropriate test systems Cr VI exposure results in direct alteration of DNA, inducing gene mutations and chromosomal alterations. The extensive literature on the mutagenicity of chromium compounds has been reviewed by a number of authors including the International Agency for Research on Cancer (1990) and De Flora *et al.* (1990). To summarize the findings, positive results were obtained *in vitro* with Cr VI compounds in gene mutation tests using *Salmonella typhimurium* or *Escherichia coli*; forward mutation and mitotic gene conversion assays in yeast; mammalian cell (including human cell lines) chromosomal damage assays that measured increases in sister chromatid exchanges, chromosomal aberrations, or micronuclei; mutation induction at the tk locus in L5178Y mouse lymphoma cells; and tests for

induction of DNA strand breaks or DNA synthesis inhibition in a variety of mammalian cells. *In vivo*, short-term assays in laboratory rodents for induction of chromosomal damage and micronuclei were positive. Comparative micronucleus assays in various mouse strains (ms, BDF1, CD-11, ddY) revealed no sex-related difference in response but did indicate differential strain and route susceptibilities to the chromosome damaging effects of Cr VI (Collaborative Study Group for the Micronucleus Test, 1986, 1988; Shindo *et al.*, 1989).

Monitoring of genetic damage endpoints in humans occupationally exposed to hexavalent chromium gave mixed results, but most studies reported observations consistent with chromium-induced chromosomal damage (IARC, 1990). For example, examination of lymphocytes of Cr VI-exposed workers revealed elevated frequencies of sister chromatid exchanges and chromosomal aberrations in several studies reviewed by IARC (1990), although some negative results were also reported. Dose-response relationships were reported for both these cytogenetic endpoints, and exposure duration was also directly correlated with effect.

Since the 1990 IARC review, results from additional cytogenetic studies in Cr VI-exposed workers have been reported, and results were positive. Vaglenov *et al.* (1999) reported increased frequencies of micronuclei in peripheral blood lymphocytes of electroplaters exposed to chromium in the workplace. They reported a direct correlation of micronucleus frequencies with chromium levels in the work atmosphere and chromium content of biological samples obtained from the workers.

In a more recent study reported by Benova *et al.* (2002), lymphocytes and exfoliated buccal cells of chromium platers in Bulgaria were examined for chromosomal aberrations, sister chromatid exchanges, and micronuclei; no increases in chromosomal aberrations or sister chromatid exchanges were seen in either cell type, but both cell types showed significant increases in micronuclei. Furthermore, in both cell types, kinetochore-positive and kinetochore-negative micronuclei were increased, suggesting that both aneuploidy and chromosomal structural damage were induced in these chromium platers (Benova *et al.*, 2002).

In another report of human exposure to hexavalent chromium, Comet analysis (single cell gel electrophoresis) of peripheral blood leukocytes of workers occupationally exposed to both chromium and nickel in a stainless steel welding facility showed significant increases in DNA damage. In addition, the frequency of micronuclei in buccal cells was elevated in the welders compared to nonexposed controls (Danadevi *et al*., 2004).

Cr VI readily enters cells, in contrast to Cr III which cannot easily pass through the cell membrane (IARC, 1990). Because it has been shown to be relatively nonreactive, Cr VI is believed to exert its genotoxic effects, at least in part, through the generation of oxygen radicals (Sugden *et al*., 1990; Sugiyama, 1992; Kasprzak, 1995; Shi *et al*., 1999;

Vaglenov *et al*., 1999; Benova *et al*., 2002; O'Brien *et al*., 2003) during metabolic transformation from the hexavalent form through the more reactive Cr V and Cr IV valences to Cr III. In studies with laboratory rodents, administration of radical scavengers simultaneously with or prior to administration of Cr VI salts reduced clastogenic potency, thus providing support for the oxygen radical mechanism of action (Chorvatovicova *et al*., 1991, 1993; Sarkar *et al*., 1993). Results of *in vitro* mammalian cell studies of Cr VI-induced DNA damage in the presence of a variety of oxygen radical scavengers and reducing agents provides additional support for this mechanism (Pattison *et al*., 2001; Cemeli *et al*., 2003; O'Brien *et al*., 2003). Cr III, the product of intracellular reduction of Cr VI, has been shown to interact directly with DNA and other macromolecules to induce chromosomal alterations and mutational changes in DNA (Zhitkovich *et al*., 2001, 2002; Quievryn *et al*., 2003). A complete understanding of the mechanisms and variables involved in Cr VI mutagenicity awaits additional study.

STUDY RATIONALE

The California congressional delegation and the California Environmental Protection Agency nominated Cr VI for toxicity and carcinogenicity testing because of concerns over its presence in drinking water supplies, its potential health effects, including carcinogenicity, and the lack of adequate carcinogenicity studies of ingested Cr VI. In response, the NTP conducted 3-month and 2-year toxicity and carcinogenicity studies of Cr VI administered in drinking water to F344/N rats and B6C3F_1 mice as sodium dichromate dihydrate. In a multigeneration study (NTP 1996a), hepatocellular vacuolization was observed in BALB/c mice fed diets providing up to 32 mg Cr VI from potassium dichromate/kg body weight per day. An additional 3-month comparative toxicity study of Cr VI was conducted with three strains (B6C3F_1, BALB/c, and *am3*-C57BL/6) of male mice to determine possible differences between strains in sensitivity to hepatotoxic effects of chromates. This report summarizes the results of toxicity studies in male and female F344/N rats and B6C3F_1 mice; comparative toxicity studies in male B6C3F_1, BALB/c, and *am3*-C57BL/6 mice; and absorption studies in F344/N rats, B6C3F_1 mice, and Hartley guinea pigs. The results of the 2-year carcinogenicity studies will be reported separately.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF SODIUM DICHROMATE DIHYDRATE

Sodium dichromate dihydrate was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (15301BI and 13822LI). The two lots were combined at the analytical chemistry laboratory, Battelle Memorial Institute (Columbus, OH), and assigned a new lot number (062001). Lot 062001 was used in the 3-month studies in F344/N rats and B6C3F$_1$ mice (study 1). An additional shipment of lot 13822LI was obtained from Aldrich Chemical Company and used in the 3-month studies in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice (study 2). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (lots 062001 and 13822LI) and by the study laboratories at Southern Research Institute (Birmingham, AL; lot 062001) and Battelle Columbus Operations (Columbus, OH; lot 13822LI). Karl Fischer titration (lots 062001 and 13822LI) and elemental analysis using inductively coupled plasma-atomic emission spectroscopy (ICP-AES; lot 062001) were performed by Galbraith Laboratories, Inc. (Knoxville, TN); elemental analysis using ICP-AES (lot 13822LI) was performed by Battelle Northwest Operations (Richland, WA); and elemental analysis using proton-induced X-ray emission spectroscopy (PIXE; lots 062001 and 13822LI) was performed by Elemental Analysis Corporation (Lexington, KY). Reports on analyses performed in support of the sodium dichromate dihydrate studies are on file at the National Institute of Environmental Health Sciences.

Lot 062001, an orange crystalline solid, was identified as sodium dichromate dihydrate by the analytical chemistry laboratory using X-ray diffraction (XRD), by the analytical chemistry laboratory and Galbraith Laboratories, Inc., using elemental analysis by ICP-AES, and by Elemental Analysis Corporation using elemental analysis by PIXE. Lot 13822LI, an orange crystalline solid, was identified as sodium dichromate dihydrate by the analytical chemistry laboratory using XRD, by the analytical chemistry laboratory and Battelle Northwest Operations using elemental analysis by ICP-AES, and by Elemental Analysis Corporation using elemental analysis by PIXE. The XRD powder patterns were consistent with a reference pattern. Elemental analyses for chromium and sodium were in agreement with the theoretical values for sodium dichromate dihydrate, and PIXE indicated the absence of significant metallic impurities.

The moisture content of lots 062001 and 13822LI was determined by Karl Fischer titration and, for lot 13822LI, weight loss on drying was performed by the analytical chemistry laboratory. The purity of lot 062001 was determined by the analytical chemistry laboratory using differential scanning calorimetry (DSC), titration of the dichromate ion with sodium thiosulfate and potassium ferrocyanide, and speciation of the chromium ions using liquid

chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) and by the study laboratory using potentiometric titrimetric analysis. The purity of lot 13822LI was determined by the analytical chemistry laboratory using DSC, titration of the dichromate ion with sodium thiosulfate and potassium ferrocyanide, and LC-ICP-MS and by the study laboratory using titration with sodium thiosulfate.

For lot 062001, Karl Fischer titration indicated a moisture content of 11.62%, which is in agreement with the theoretical value of 12.09%. DSC indicated a purity of 99.73% ± 0.15%. Titration with sodium thiosulfate by the analytical chemistry laboratory indicated a purity of 99.7% ± 0.1%. Titration with sodium thiosulfate by the study laboratory indicated purities of 101% and 102% relative to a frozen reference standard of the same lot. Titration with potassium ferrocyanide indicated a purity of 103.1% ± 0.2%. LC-ICP-MS indicated that the concentration of Cr III, if present, was less than 0.1%. The overall purity of lot 062001 was determined to be greater than 99.7%.

For lot 13822LI, Karl Fischer titration indicated a moisture content of 9.16%, less than the theoretical value of 12.09%; however, the percentage weight loss on drying agreed well with the theoretical value. DSC indicated a purity of 99.10% ± 0.27%. Titration with sodium thiosulfate and potassium ferrocyanide at the analytical chemistry laboratory indicated purities of 99.1% ± 1.2% and 99.6% ± 1.6%, respectively. Titration with sodium thiosulfate at the study laboratory indicated a purity of 101.8%. LC-ICP-MS indicated that the concentration of Cr III, if present, was less than 0.1%. The overall purity of lot 13822LI was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at room temperature, protected from light in amber glass bottles (lot 062001) or in a white plastic bottle (lot 13822LI). During the studies, stability of the bulk chemical was monitored by the study laboratories using potentiometric titration (lot 062001) or titration of the dichromate ion with sodium thiosulfate (lot 13822LI). No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared four times during the 3-month studies in F344/N rats and B6C3F$_1$ mice (study 1) and five times during the 3-month studies in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice (study 2). Formulations used in study 1 were stored in NALGENE® containers at room temperature and protected from light. Formulations used in study 2 were stored in NALGENE® containers and refrigerated at approximately 5° C.

Stability studies of a 41.8 µg/mL sodium dichromate dihydrate dose formulation were performed by the analytical chemistry laboratory using ion chromatography (IC). Stability was confirmed for at least 42 days for dose formulations stored in sealed NALGENE® containers, protected from light, at temperatures up to room temperature and for at least 7 days when stored in drinking water bottles under simulated animal room conditions.

Periodic analyses of the dose formulations of sodium dichromate dihydrate were conducted during study 1 by the study laboratory using ultraviolet spectroscopy and during study 2 by the analytical chemistry laboratory using IC. During study 1, the dose formulations were analyzed three times. All 15 of the dose formulations for rats and mice were within 10% of the target concentrations. Animal room samples and unused carboy storage samples of these dose formulations were also analyzed; 14 of 15 animal room samples for rats and all 15 of the animal room samples for mice were within 10% of target concentrations. All 15 of the unused carboy samples were within 10% of the target concentrations. During study 2, the dose formulations were analyzed three times. All nine of the dose formulations for mice were within 10% of the target concentrations. Animal room samples and unused carboy storage samples of the these dose formulations were also analyzed; all nine of the animal room samples and all nine of the carboy samples were within 10% of the target concentrations.

3-Month Studies in Male and Female F344/N Rats and B6C3F$_1$ Mice (Study 1)

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 to 14 days and were 5 to 7 weeks old on the first day of the study. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Blood was collected from five male and five female core study control rats and mice at study termination. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al*., 1986; Rao *et al*., 1989a,b). All results were negative.

Groups of 10 male and 10 female F344/N rats and 10 male and 10 female B6C3F$_1$ mice were exposed to sodium dichromate dihydrate in drinking water at concentrations of 0, 62.5, 125, 250, 500, or 1,000 mg/L. In addition, groups of 10 male and 10 female rats for clinical pathology studies were exposed to the same concentrations for 4 weeks. Water consumption by core study animals was recorded weekly by cage. All animals were weighed initially; core study animals were weighed weekly and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Blood was collected from the retroorbital sinus of clinical pathology rats on days 5 and 23 and from core study rats and mice at the end of the study for hematology and clinical chemistry (rats) analyses. On day 16, clinical pathology rats were placed into metabolism cages, and urine was collected over ice for approximately 24 hours. Blood for hematology was collected from each animal into a tube containing EDTA, and for clinical chemistry analyses, blood was collected from each rat into tubes containing no anticoagulant. Hematology analyses were performed within approximately 6 hours; clinical chemistry analyses were completed within 8 hours after blood collection. Hematology reagents were supplied by Bayer, Inc. (Tarrytown, NY), or Fischer Scientific (Norcross, GA).

Hematology analyses and reticulocyte counts were conducted on the day of sample collection using an ADVIA 120 Hematology System Analyzer (Boehringer Mannheim Corp., Indianapolis, IN). Clinical chemistry analyses were conducted on a Hitachi 911 Clinical Chemistry Analyzer (Boehringer Mannheim Corp., Indianapolis, IN). Clinical chemistry reagents were supplied by Sigma Diagnostics (St. Louis, MO), Roche Diagnostics (Indianapolis, IN), or Boehringer Mannheim Corp. Blood smears were prepared within approximately 2 hours of sample collection for evaluation of platelet and erythrocyte morphology by light microscopy. Urinalysis reagents were supplied by Bayer, Inc., or Hycor Biomedical (Irvine, CA). The parameters measured are listed in Table 1.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, spleen, right testis, and thymus of all core study animals were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin with the exception of eyes, which were initially fixed in Davidson's solution, then transferred to 10% neutral buffered formalin approximately 24 hours after collection. Tissues were processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 µm and stained with hematoxylin and eosin. Complete histopathological examinations were performed on all core study animals in the 0 and 1,000 mg/L groups and on 6 of 10 randomly selected rats and mice in each of the other exposed groups. Tissues identified as target organs in the 1,000 mg/L group were examined in lower exposure concentration groups until a no-effect level had been determined or all animals had been examined. Table 1 lists the tissues and organs routinely examined.

3-Month Comparative Toxicity Studies in Male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 Mice (Study 2)

Three strains (B6C3F$_1$, BALB/c, and *am3*-C57BL/6) of male mice were selected as the test system for the 3-month comparative toxicity studies of sodium dichromate dihydrate in mice. The mice were received in three strain-specific shipments all within the same week. This study evaluated potential strain-specific liver responses to chromate exposure.

The B6C3F$_1$ mice were supplied by Taconic Laboratory Animals and Services (Germantown, NY), the BALB/c mice were supplied by Charles River Laboratory (Portage, MI), and the *am3*-C57BL/6 mice were supplied by Charles River Laboratory (Wilmington, MA). All mice were approximately 4 weeks old at receipt. B6C3F$_1$ mice were quarantined for 12 days, BALB/c mice for 16 days, and *am3*-C57BL/6 mice for 17 days. B6C3F$_1$ and BALB/c mice were 6 weeks old when placed on study, and *am3*-C57BL/6 mice were 7 weeks old.

All three strains of mice were housed and quarantined in the same room. Before the study began, serum samples for viral titer testing were collected from five mice per strain not selected for study; these animals were euthanized, necropsied, and examined grossly for the presence of disease or parasites. Serum samples were also collected from

five sentinel mice per strain approximately 4 weeks after quarantine release and at study termination from 10 sentinel mice per strain. Sera were analyzed for antibody titers to rodent viruses. All results were negative.

In the core study, groups of 10 male B6C3F$_1$, 10 male BALB/c, and five male *am3*-C57BL/6 mice received sodium dichromate dihydrate in drinking water at concentrations of 0, 62.5, 125, or 250 mg/L for 3 months. A top sodium dichromate dihydrate concentration of 250 mg/L in drinking water was chosen based on the poor palatability of higher concentrations in the first study with B6C3F$_1$ mice. Additional groups of five male *am3*-C57BL/6 mice were exposed to the same concentrations for 3 months for a mutagenicity study. The *am3*-C57BL/6 strain is a C57BL/6 mouse transgenic for the phiX174*am3* gene. This is a nonessential integrated transgene that provides a target for forward and backward mutations and can be recovered from cells and scored for mutation frequency and type (Malling and Burkhart, 1989). Due to technical difficulties, these mutation studies could not be completed, and no results are included in this Toxicity Study Report.

Clinical findings and body weights were recorded for all mice on study day 1, weekly thereafter, and at study termination. Water consumption was measured at least every 4 days. Details of the study design and animal maintenance are summarized in Table 1.

Blood samples for clinical pathology determinations were collected by cardiac puncture from core study mice and up to five mutagenicity study mice per exposure group at study termination. Blood for hematology evaluations was placed in microcollection tubes containing potassium EDTA; samples for clinical chemistry determinations were placed in serum collection tubes and centrifuged. The time blood first entered the syringe was recorded for B6C3F$_1$ mice as part of the RNA procedure.

At the end of the 3-month studies, samples were collected for sperm count and motility from all core study mice. The parameters evaluated are listed in Table 1. 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. Modified Tyrode's buffer 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 mice at study termination. Organ weights from core study mice were recorded for the heart, right kidney, liver (except $B6C3F_1$ mice), lung, spleen, right testis, and thymus. Tissues for microscopic examination were processed as described for study 1. The liver, forestomach, glandular stomach, small intestine (duodenum), pancreas, kidney, and mesenteric and pancreatic lymph nodes of core study mice were examined for histopathology. A complete histopathologic evaluation was not performed in this study.

Upon completion of the laboratory pathologists' histopathologic evaluations, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to independent pathology laboratories where quality assessments were performed. Results were reviewed and evaluated by the NTP Pathology Working Groups (PWGs); the final diagnoses represent a consensus of contractor pathologists and the PWGs. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

TABLE 1
Experimental Design and Materials and Methods in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate

F344/N Rats and B6C3F$_1$ Mice (Study 1)	B6C3F$_1$, BALB/c, and *am3*-C57BL/6 Mice (Study 2)
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Battelle Columbus Operations (Columbus, OH)
Strain and Species	
F344/N rats B6C3F$_1$ mice	B6C3F$_1$ mice BALB/c mice *am3*-C57BL/6 mice
Animal Source	
Taconic Farms, Inc. (Germantown, NY)	B6C3F$_1$: Taconic Laboratory Animals and Services (Germantown, NY) BALB/c: Charles River Laboratory (Portage, MI) *am3*-C57BL/6: Charles River Laboratory (Wilmington, MA)
Time Held Before Study	
Rats: 12 days (males) or 11 days (females) Mice: 13 days (males) or 14 days (females)	B6C3F$_1$: 12 days BALB/c: 16 days *am3*-C57BL/6: 17 days
Average Age When Study Began	
5 to 7 weeks	6 (B6C3F$_1$ and BALB/c mice) or 7 (*am3*-C57BL/6 mice) weeks
Date of First Exposure	
Rats: November 12, 2001 (males) November 11, 2001 (females) Mice: November 13, 2001 (males) November 14, 2001 (females)	B6C3F$_1$: August 20, 2002 BALB/c: August 22, 2002 *am3*-C57BL/6: August 23, 2002
Duration of Exposure	
Rats: 14 weeks (core), 4 weeks (clinical pathology study) Mice: 14 weeks	14 weeks
Date of Last Exposure	
Rats: February 12, 2002 (males, core study) February 11, 2002 (females, core study) December 4, 2001 (males, clinical pathology study) December 3, 2001 (females, clinical pathology study) Mice: February 13, 2002 (males) February 14, 2002 (females)	B6C3F$_1$: November 19, 2002 BALB/c: November 21, 2002 *am3*-C57BL/6: November 22, 2002
Necropsy Dates	
Rats: February 12, 2002 (males, core study) February 11, 2002 (females, core study) Mice: February 13, 2002 (males) February 14, 2002 (females)	B6C3F$_1$: November 19, 2002 BALB/c: November 21, 2002 *am3*-C57BL/6: November 22, 2002
Average Age at Necropsy	
Rats: 18 to 19 weeks (core study) Mice: 19 to 20 weeks	19 (B6C3F$_1$ and BALB/c mice) or 20 (*am3*-C57BL/6 mice) weeks
Size of Study Groups	
10 males and 10 females	10 males (B6C3F$_1$ and BALB/c mice) 5 males (*am3*-C57BL/6 mice, core study) 5 males (*am3*-C57BL/6 mice, mutagenicity study

TABLE 1
Experimental Design and Materials and Methods in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate

F344/N Rats and B6C3F$_1$ Mice (Study 1)	B6C3F$_1$, BALB/c, and *am3*-C57BL/6 Mice (Study 2)
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as study 1
Animals per Cage Rats: 5 Mice: 1 (males) or 5 (females)	1
Method of Animal Identification Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 wafer rodent feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as study 1
Water Tap water (Birmingham, AL municipal supply) via amber glass water bottles with Teflon®-lined caps and stainless steel sipper tubes (Wheaton, Millville, NJ), available *ad libitum*	Tap water (Columbus, OH, municipal supply) via glass bottles with Teflon®-lined septa and stainless steel sipper tubes (Wheaton, Millville, NJ), available *ad libitum*
Cages Solid bottom polycarbonate (Lab Products, Inc., Maywood, NJ), changed at least twice weekly	Polycarbonate (Lab Products, Inc., Maywood, NJ), changed at least once weekly
Bedding Irradiated hardwood bedding chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed at least twice weekly	Irradiated hardwood bedding chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed at least once weekly
Cage Filters Reemay spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Spun-bonded polyester (Snow Filtration Co., Cincinnati, OH)
Racks Stainless Steel (Lab Products, Inc., Maywood, NJ), changed every 2 weeks	Stainless Steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 18/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 10/hour
Exposure Concentrations 0, 62.5, 125, 250, 500, or 1,000 mg/L in drinking water, available *ad libitum*	0, 62.5, 125, or 250 mg/L in drinking water, available *ad libitum*
Type and Frequency of Observation Observed twice daily; core study animals were weighed initially, weekly, and at the end of the study; clinical findings were recorded weekly; and water consumption was measured weekly. Clinical pathology study rats were weighed on day 1.	Observed twice daily. Body weights and clinical findings were recorded on day 1, weekly thereafter, and at the end of the study. Water consumption was measured at least every 4 days.
Method of Sacrifice CO_2 asphyxiation	CO_2 asphyxiation

TABLE 1
Experimental Design and Materials and Methods in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate

F344/N Rats and B6C3F$_1$ Mice (Study 1)	B6C3F$_1$, BALB/c, and *am3*-C57BL/6 Mice (Study 2)
Necropsy	
Necropsies were performed on all core study rats and mice. Organs weighed were heart, right kidney, liver, lung, spleen, right testis, and thymus.	Necropsies were performed on all core study mice. Organs weighed were heart, right kidney, liver (except B6C3F$_1$ mice) lung, spleen, right testis, and thymus.
Clinical Pathology	
Blood was collected from the retroorbital sinus of clinical pathology study rats on days 5 and 23 and core study rats and mice at the end of the studies for hematology and clinical chemistry (rats). Clinical pathology study rats were placed in metabolism cages on day 16 for 24-hour urine collection. ***Hematology:*** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, platelet counts, and platelet estimates; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, glucose, sodium, potassium, chloride, calcium, phosphorus, total protein, albumin, cholesterol, triglycerides, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, 5′-nucleotidase, total bile acids ***Urinalysis:*** creatinine, glucose, protein, alkaline phosphatase, aspartate aminotransferase, N-acetyl-glucosaminidase, volume, specific gravity, pH	Blood was collected by cardiac puncture at the end of the studies from all core study mice and up to five mutagenicity study mice for hematology and clinical chemistry. ***Hematology:*** automated and manual hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, nucleated erythrocyte, platelet counts, and platelet estimates; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, total bile acids
Histopathology	
Complete histopathology was performed on 0 and 1,000 mg/L core study rats and mice and on 6 of 10 randomly selected rats and mice in each of the other exposed groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, clitoral gland, esophagus, eye, harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular, mesenteric, and pancreatic), 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 and vaginal tunics, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Histopathology was performed on all core study mice. In addition to gross lesions and tissue masses, the liver, forestomach, glandular stomach, small intestine (duodenum), pancreas, kidney, and mesenteric and pancreatic lymph nodes were examined.
Sperm Motility Evaluation	
None	Sperm count and motility were performed on all core study animals. The following parameters were examined: spermatids per testis and per mg testis, spermatids per cauda and per mg cauda, and sperm motility. The left cauda, left epididymis, and left testis were weighed.

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 (Gart *et al.,* 1979), 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 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 (1955) and Williams (1971, 1972). Hematology, clinical chemistry, urinalysis, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the exposure concentration-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 exposure concentration-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations.

QUALITY ASSURANCE METHODS

The 3-month studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Units of Southern Research Institute (study 1) and Battelle Columbus Operations (study 2) performed audits and inspections of protocols, procedures, data, and reports throughout the course of the study.

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Testing was performed essentially as reported by Zeiger *et al.* (1992), with modifications described in the brief summary presented here. Sodium dichromate dihydrate was sent to the testing laboratory as a coded aliquot. Test concentrations of sodium dichromate dihydrate were dissolved in water and preincubated with bacterial tester strains

(*Salmonella typhimurium* TA98 and TA100 and *Escherichia coli* WP2 uvrA pKM101) either in buffer or 10% S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat 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 at least five doses of sodium dichromate dihydrate. The high dose was limited by toxicity. All trials were repeated.

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.

Mouse Peripheral Blood Micronucleus Test Protocol

Two independent studies were conducted to evaluate the effect of sodium dichromate dihydrate, administered in drinking water for 3 months, on the frequency of micronuclei in peripheral blood erythrocytes of mice. A detailed discussion of this assay is presented by MacGregor *et al.* (1990). In study 1, male and female B6C3F$_1$ mice were administered sodium dichromate dihydrate over an exposure concentration range of 62.5 to 1,000 mg/L for 3 months. In study 2, micronucleus frequencies were evaluated in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice administered sodium dichromate dihydrate over an exposure concentration range of 62.5 to 250 mg/L in drinking water for 3 months. At the ends of the 3-month exposure periods, peripheral blood samples were obtained from mice, and 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 micronucleated cells in 2,000 normochromatic erythrocytes (NCEs) in each of five mice per treatment group for all except the *am3*-C57BL/6 strain; for the *am3*-C57BL/6 mice, five core study and four or five mutagenicity study mice per treatment group were evaluated. In addition to assessment of micronucleus frequencies, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within an exposure group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs and PCEs 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 (ILS, 1990). In the

presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Because additional test data could not be obtained, results of the 3-month studies were accepted without repeat tests. 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 aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The results presented in the Abstract of this Toxicity Study Report represent a scientific judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

STUDY 1
3-MONTH STUDY IN MALE AND FEMALE F344/N RATS

Administration of sodium dichromate dihydrate in the drinking water had no effect on survival of male or female rats but produced mild deficits in body weight gain for male and female rats exposed to 1,000 mg/L (Table 2, Figure 1). The final mean body weights of male and female rats in the 1,000 mg/L group were 89% and 94%, respectively, of the final mean body weights of male and female control rats; the final mean body weights and body weight gain of 500 mg/L males were also less than those of the controls. Water consumption by male and female rats in the 250, 500, and 1,000 mg/L groups was less than that by the controls. Exposure concentrations of 62.5, 125, 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 5, 9, 17, 32, and 60 mg/kg body weight to males and 5, 10, 18, 33, and 61 mg/kg to females. No clinical findings were attributed to sodium dichromate dihydrate exposure.

TABLE 2
Survival, Body Weights, and Water Consumption of Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	101 ± 1	316 ± 7	215 ± 6		15.9	14.8
62.5	10/10	100 ± 2	296 ± 4	196 ± 4	94	15.6	12.0
125	10/10	101 ± 1	324 ± 6	223 ± 5	103	14.7	14.0
250	10/10	101 ± 1	323 ± 3	222 ± 3	102	13.8	12.7
500	10/10	101 ± 1	301 ± 3*	200 ± 2*	95	11.9	13.4
1,000	10/10	101 ± 1	283 ± 5**	181 ± 4**	89	9.0	11.6
Female							
0	10/10	89 ± 1	190 ± 3	101 ± 3		14.1	11.2
62.5	10/10	91 ± 1	205 ± 3	114 ± 3	108	14.0	12.7
125	10/10	89 ± 1	195 ± 2	107 ± 3	103	13.0	10.9
250	10/10	90 ± 1	193 ± 2	103 ± 2	102	11.7	9.4
500	10/10	89 ± 1	189 ± 3	100 ± 3	100	10.3	9.1
1,000	10/10	88 ± 1	179 ± 2**	91 ± 2*	94	8.3	8.1

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

** $P \leq 0.01$

[a] Number of animals surviving at 3 months/number initially in group

[b] Weights and weight changes are given as mean ± standard error.

[c] Water consumption is expressed as grams per animal per day.

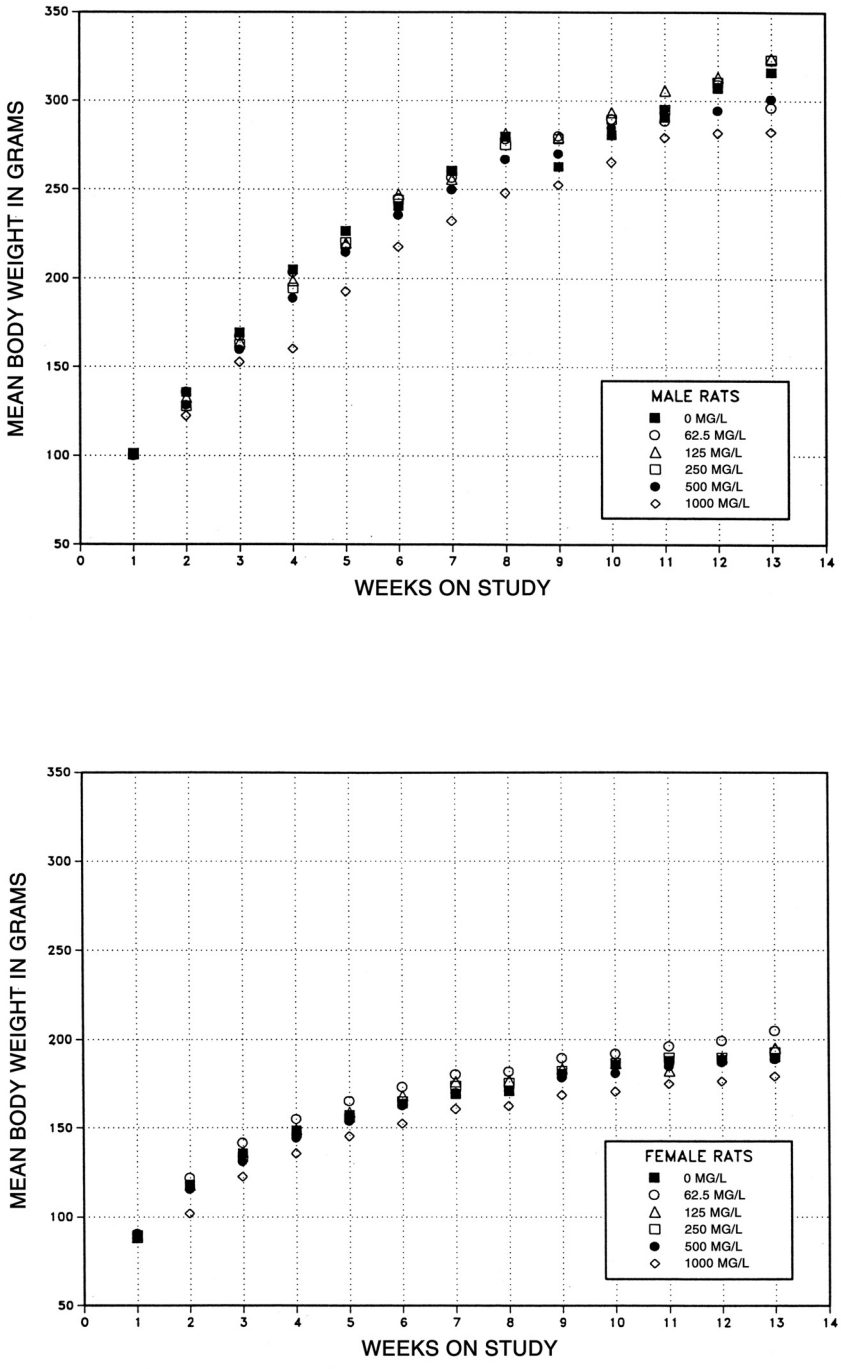


FIGURE 1
Body Weights of Rats Administered Sodium Dichromate Dihydrate in Drinking Water for 3 Months

The hematology and clinical chemistry data are listed in Tables 3 and C1. The most dramatic effect of sodium dichromate dihydrate administration involved the erythron. An exposure-related microcytic, hypochromic, responsive anemia occurred in exposed rats.

The microcytosis, evidenced by decreased mean cell volumes, occurred at day 5 and persisted throughout the study in all exposed groups. In 1,000 mg/L rats, the severity of the microcytosis was unchanged in females and increased with time in males; and, at week 14, erythrocytes in 1,000 mg/L rats were approximately 30% and 25% smaller in males and females, respectively. At lower exposure concentrations, microcytosis was most pronounced on day 23 (approximately 25% smaller in 500 mg/L males and females) and, in general, ameliorated with time.

The anemia, evidenced by decreases in automated and manual hematocrit values, hemoglobin concentrations, and erythrocyte counts, developed in all exposed groups by day 23 and persisted to week 14; it was most severe at day 23 and ameliorated with time. At week 14, erythrocyte counts were increased and contradictory to the lower hematocrit values and hemoglobin concentrations. The increased numbers of reticulocytes and nucleated erythrocytes were indicative of an erythropoietic response. Thus, while there was an apparent erythropoietic response resulting in increased numbers of circulating erythrocytes, the erythrocytes produced were smaller, which resulted in a decreased erythron in the 250 mg/L or greater groups at week 14. Microscopic evaluation of the blood smears demonstrated increased erythrocyte fragments, keratocytes, and blebbing that suggested increased erythrocyte injury or turnover. Additionally, increased numbers of hypochromic microcytes were observed suggesting that blood loss or altered iron metabolism or hemoglobin production was involved (Plate 1). Gastric ulcers may have resulted in blood loss, but this lesion was only seen in the 1,000 mg/L groups, and the hypochromic microcytosis occurred in most exposed animals. Thus, some alteration in iron metabolism or hemoglobin production was suspected.

Normally, instrument-derived hematocrit values should closely approximate the manual hematocrit value, which is considered the "gold standard" method. However, in this study, the instrument-derived values were noticeably lower than the manual hematocrit values in the 125 mg/L or greater groups at day 23 and in the 1,000 mg/L males at week 14. Microscopic evaluations of the blood smears demonstrated increased numbers of microcytic erythrocyte fragments, keratocytes, and hypochromic microcytes. The decreased instrument-derived hematocrit values could possibly be explained by the instrument's inability to recognize and count the small erythrocytes or erythrocyte fragments or adequately determine the erythrocyte size (mean cell volume). Because mean cell hemoglobin concentration (MCHC) is calculated using the instrument-derived hematocrit values, a decrease in these hematocrit values would result in increased MCHCs (Table 3). However, recalculated mean MCHCs using the mean manual hematocrit values were decreased compared to control MCHC values and suggested a hypochromia (iron deficiency-like process).

TABLE 3
Selected Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
Hematology						
n	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 5	45.8 ± 1.0	45.0 ± 0.8	45.2 ± 0.9	43.8 ± 0.8	44.6 ± 0.6	46.2 ± 0.7
Day 23	48.5 ± 0.7	45.0 ± 1.0*	34.3 ± 1.8**	28.0 ± 1.4**	24.3 ± 0.9**	21.1 ± 1.6**
Week 14	46.0 ± 0.3	45.5 ± 0.4	45.3 ± 0.3	44.9 ± 0.7	43.1 ± 0.5**	30.8 ± 1.9**
Hematocrit (manual) (%)						
Day 5	45.6 ± 1.1	45.1 ± 0.7	45.0 ± 0.8	44.0 ± 0.8	44.5 ± 0.6	45.9 ± 0.7
Day 23	48.0 ± 0.5	44.7 ± 0.7**	39.8 ± 0.8**	36.2 ± 1.0**	34.4 ± 0.5**	32.3 ± 1.1**
Week 14	45.7 ± 0.2	45.2 ± 0.4	45.2 ± 0.3	44.8 ± 0.7	42.9 ± 0.4**	36.9 ± 0.8**
Hemoglobin (g/dL)						
Day 5	15.3 ± 0.4	15.0 ± 0.2	15.1 ± 0.3	14.9 ± 0.3	15.1 ± 0.2	15.7 ± 0.2
Day 23	15.9 ± 0.1	14.2 ± 0.2**	12.0 ± 0.3**	10.9 ± 0.3**	10.3 ± 0.3**	9.2 ± 0.3**
Week 14	15.3 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	14.4 ± 0.2**	13.3 ± 0.2**	10.9 ± 0.3**
Erythrocytes (10^6/μL)						
Day 5	7.27 ± 0.17	7.30 ± 0.12	7.46 ± 0.14	7.36 ± 0.13	7.43 ± 0.10	7.70 ± 0.10
Day 23	7.94 ± 0.10	8.38 ± 0.11	7.13 ± 0.35*	6.03 ± 0.28**	5.25 ± 0.19**	4.54 ± 0.33**
Week 14	8.88 ± 0.05	9.04 ± 0.09*	9.25 ± 0.07**	10.15 ± 0.22**	10.87 ± 0.07**	8.52 ± 0.45**
Reticulocytes (10^6/μL)						
Day 5	0.63 ± 0.02	0.52 ± 0.01**	0.31 ± 0.02**	0.19 ± 0.02**	0.17 ± 0.03**	0.10 ± 0.01**
Day 23	0.26 ± 0.01	0.40 ± 0.01**	0.39 ± 0.03**	0.32 ± 0.02	0.27 ± 0.01	0.27 ± 0.02
Week 14	0.23 ± 0.00	0.24 ± 0.00	0.22 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.31 ± 0.01**
Nucleated erythrocytes (10^3/μL)						
Day 5	0.20 ± 0.13	0.30 ± 0.15	0.20 ± 0.13	0.30 ± 0.15	0.10 ± 0.10	0.40 ± 0.16
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.70 ± 0.26**	1.30 ± 0.40**	1.90 ± 0.64**	2.70 ± 0.78**
Week 14	0.10 ± 0.10	0.30 ± 0.15	0.10 ± 0.10	0.10 ± 0.10	0.20 ± 0.13	1.20 ± 0.29**
Mean cell volume (fL)						
Day 5	63.1 ± 0.2	61.7 ± 0.2**	60.6 ± 0.2**	59.5 ± 0.2**	60.0 ± 0.2**	60.0 ± 0.3**
Day 23	61.1 ± 0.5	53.6 ± 0.6**	48.0 ± 0.4**	46.4 ± 0.6**	46.2 ± 0.3**	46.4 ± 0.5**
Week 14	51.8 ± 0.1	50.3 ± 0.2**	49.0 ± 0.1**	44.4 ± 1.0**	39.7 ± 0.5**	36.0 ± 0.4**
Mean cell hemoglobin (pg)						
Day 5	21.0 ± 0.1	20.5 ± 0.1**	20.3 ± 0.1**	20.3 ± 0.1**	20.4 ± 0.1**	20.4 ± 0.1**
Day 23	20.1 ± 0.2	16.9 ± 0.2**	17.2 ± 0.7**	18.2 ± 0.4	19.7 ± 0.3	20.7 ± 0.6
Week 14	17.3 ± 0.1	16.9 ± 0.1**	16.2 ± 0.1**	14.2 ± 0.4**	12.3 ± 0.2**	13.0 ± 0.5**
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.3 ± 0.2	33.3 ± 0.2	33.5 ± 0.1	34.1 ± 0.1**	34.0 ± 0.1**	34.0 ± 0.1**
Day 23	32.9 ± 0.4	31.6 ± 0.3	35.9 ± 1.7	39.2 ± 0.9**	42.8 ± 0.7**	44.6 ± 1.3**
Week 14	33.4 ± 0.1	34.0 ± 0.1	33.1 ± 0.2	32.0 ± 0.2**	31.0 ± 0.2**	36.3 ± 1.8*
Platelets (10^3/μL)						
Day 5	913.6 ± 28.9	943.0 ± 21.7	1,084.0 ± 51.6**	1,222.2 ± 46.4**	1,239.0 ± 43.2**	1,286.8 ± 36.2**
Day 23	745.2 ± 22.2	1,065.3 ± 67.9**	2,768.6 ± 328.5**	3,504.7 ± 235.0**	4,226.0 ± 204.5**	4,688.8 ± 242.7**
Week 14	618.6 ± 20.0	736.1 ± 11.5	604.3 ± 24.5	909.8 ± 119.1**	1,743.1 ± 178.0**	5,123.0 ± 638.9**
Platelet estimates (10^3/μL)						
Day 23	1,302.0 ± 68.9	1,537.2 ± 102.7	1,957.2 ± 106.2**	1,900.5 ± 170.3**	1,917.3 ± 83.7**	2,083.2 ± 158.5**
Week 14	676.2 ± 32.8	674.1 ± 25.3	636.3 ± 24.1	663.6 ± 21.5	678.3 ± 29.2	783.3 ± 28.5

TABLE 3
Selected Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (continued)						
Clinical Chemistry						
n	10	10	10	10	10	10
Cholesterol (mg/dL)						
Day 5	112 ± 3	106 ± 2	103 ± 2**	103 ± 1**	103 ± 2**	103 ± 3**
Day 23	86 ± 3	69 ± 2**	78 ± 2	76 ± 2	71 ± 1**	77 ± 2
Week 14	89 ± 2	95 ± 2	86 ± 4	65 ± 2**	86 ± 3*	71 ± 2**
Triglycerides (mg/dL)						
Day 5	119 ± 9	111 ± 11	110 ± 7	119 ± 6	109 ± 8	112 ± 10
Day 23	212 ± 12	168 ± 7	191 ± 9	157 ± 16**	146 ± 7**	109 ± 7**
Week 14	170 ± 9	169 ± 8	172 ± 15	170 ± 13	164 ± 12	98 ± 8**
Alanine aminotransferase (IU/L)						
Day 5	48 ± 1	55 ± 2**	69 ± 2**	73 ± 3**	73 ± 3**	70 ± 2**
Day 23	44 ± 1	63 ± 5**	65 ± 2**	69 ± 2**	75 ± 3**	67 ± 3**
Week 14	98 ± 6[b]	274 ± 30**	461 ± 102**	447 ± 121**	740 ± 81**	191 ± 17**
Creatine kinase (IU/L)						
Day 5	407 ± 23	434 ± 49	495 ± 30	486 ± 36	533 ± 43**	520 ± 21**
Day 23	586 ± 35	582 ± 62	663 ± 67	636 ± 74[b]	810 ± 73	656 ± 82
Week 14	214 ± 26	286 ± 32	291 ± 36	364 ± 23**	413 ± 16**	374 ± 44**
Sorbitol dehydrogenase (IU/L)						
Day 5	19 ± 1	20 ± 1	17 ± 1	18 ± 1	17 ± 1	17 ± 1
Day 23	20 ± 2	16 ± 1	16 ± 1	16 ± 2	16 ± 1	16 ± 1
Week 14	31 ± 2[b]	55 ± 5**	110 ± 24**	102 ± 24**	173 ± 20**	59 ± 6**
Bile acids (µmol/L)						
Day 5	29.0 ± 1.6	25.9 ± 1.8	26.6 ± 1.6	32.1 ± 1.9	28.0 ± 1.7	26.5 ± 1.8
Day 23	23.4 ± 1.6	23.2 ± 2.4	29.1 ± 1.7	30.4 ± 3.1	29.8 ± 2.2	32.8 ± 1.6**
Week 14	22.0 ± 2.2	24.0 ± 3.4	34.5 ± 7.0	32.6 ± 5.3	45.3 ± 2.8**	28.1 ± 2.0**
Female						
Hematology						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	9	10	10	10	9
Hematocrit (automated) (%)						
Day 5	48.2 ± 1.3	48.4 ± 0.8	47.4 ± 1.3	46.8 ± 1.2	48.7 ± 0.6	48.5 ± 1.0
Day 23	47.7 ± 0.4	45.9 ± 0.9	35.2 ± 1.1**	29.6 ± 2.0**	24.1 ± 1.2**	19.5 ± 0.7**
Week 14	44.2 ± 0.3	45.8 ± 0.2	44.0 ± 0.2	42.8 ± 0.3*	42.8 ± 0.4*	38.4 ± 0.6**
Hematocrit (manual) (%)						
Day 5	47.8 ± 1.1	48.5 ± 0.8	47.2 ± 1.3	46.3 ± 1.3	48.3 ± 0.6	47.7 ± 1.0
Day 23	48.0 ± 0.4	46.6 ± 0.9	42.9 ± 0.8**	39.2 ± 0.7**	37.2 ± 0.7**	33.4 ± 0.6**
Week 14	44.6 ± 0.4	45.2 ± 0.1	44.1 ± 0.3	42.9 ± 0.2**	42.6 ± 0.5**	38.3 ± 0.5**
Hemoglobin (g/dL)						
Day 5	16.1 ± 0.4	16.2 ± 0.3	15.9 ± 0.4	15.7 ± 0.4	16.3 ± 0.2	16.4 ± 0.3
Day 23	15.9 ± 0.1	14.7 ± 0.3**	13.0 ± 0.3**	11.8 ± 0.3**	10.9 ± 0.2**	9.7 ± 0.2**
Week 14	15.2 ± 0.1	15.4 ± 0.1	14.9 ± 0.1	14.3 ± 0.1**	14.1 ± 0.2**	12.0 ± 0.2**

TABLE 3
Selected Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Hematology (continued)						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	9	10	10	10	9
Erythrocytes (10^6/μL)						
Day 5	7.66 ± 0.21	7.77 ± 0.13	7.74 ± 0.20	7.68 ± 0.17	8.00 ± 0.08	8.03 ± 0.15
Day 23	7.82 ± 0.09	8.52 ± 0.14	7.22 ± 0.19	6.32 ± 0.36**	5.27 ± 0.23**	4.21 ± 0.16**
Week 14	8.30 ± 0.06	8.60 ± 0.05**	8.40 ± 0.04*	8.47 ± 0.04*	8.93 ± 0.11**	9.62 ± 0.10**
Reticulocytes (10^6/μL)						
Day 5	0.50 ± 0.02	0.43 ± 0.02*	0.33 ± 0.02**	0.22 ± 0.03**	0.24 ± 0.03**	0.12 ± 0.01**
Day 23	0.21 ± 0.01	0.27 ± 0.02	0.32 ± 0.03*	0.30 ± 0.03*	0.23 ± 0.02	0.22 ± 0.03
Week 14	0.17 ± 0.00	0.22 ± 0.01**	0.21 ± 0.00**	0.21 ± 0.01**	0.21 ± 0.01**	0.24 ± 0.02**
Nucleated erythrocytes (10^3/μL)						
Day 5	0.20 ± 0.13	0.30 ± 0.15	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.33 ± 0.24	0.38 ± 0.18	0.33 ± 0.17	0.40 ± 0.16	1.11 ± 0.39**
Week 14	0.30 ± 0.21	0.11 ± 0.11	0.40 ± 0.22	0.20 ± 0.20	0.00 ± 0.00	0.11 ± 0.11
Mean cell volume (fL)						
Day 5	63.0 ± 0.3	62.3 ± 0.3	61.2 ± 0.3**	60.8 ± 0.4**	60.9 ± 0.3**	60.4 ± 0.3**
Day 23	61.1 ± 0.4	53.9 ± 0.5**	48.8 ± 0.5**	46.6 ± 0.6**	45.7 ± 0.4**	46.5 ± 0.5**
Week 14	53.3 ± 0.1	53.3 ± 0.1	52.4 ± 0.2**	50.5 ± 0.3**	48.0 ± 0.9**	40.0 ± 0.7**
Mean cell hemoglobin (pg)						
Day 5	21.0 ± 0.1	20.8 ± 0.1	20.5 ± 0.1*	20.4 ± 0.1**	20.4 ± 0.1**	20.5 ± 0.1**
Day 23	20.4 ± 0.1	17.3 ± 0.2	18.0 ± 0.3	18.9 ± 0.7	21.0 ± 0.6	23.1 ± 0.5
Week 14	18.4 ± 0.1	17.9 ± 0.1**	17.8 ± 0.1**	16.9 ± 0.1**	15.9 ± 0.4**	12.5 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.4 ± 0.2	33.4 ± 0.2	33.5 ± 0.2	33.6 ± 0.2	33.5 ± 0.2	33.9 ± 0.2
Day 23	33.3 ± 0.1	32.1 ± 0.1	37.0 ± 0.7*	40.8 ± 1.8**	45.8 ± 1.4**	49.6 ± 0.9**
Week 14	34.5 ± 0.1	33.7 ± 0.1**	33.9 ± 0.1**	33.5 ± 0.1**	33.0 ± 0.3**	31.2 ± 0.2**
Platelets (10^3/μL)						
Day 5	856.8 ± 36.4	872.1 ± 20.5	958.3 ± 34.2	1,045.8 ± 47.5*	1,003.3 ± 45.1*	1,002.1 ± 41.5*
Day 23	611.5 ± 43.7	1,156.3 ± 76.4**	2,808.8 ± 198.5**	3,295.0 ± 349.7**	4,318.4 ± 234.9**	5,132.8 ± 247.0**
Week 14	588.9 ± 17.1	605.8 ± 17.1	574.8 ± 21.3	528.2 ± 14.1	619.3 ± 55.4	1,524.9 ± 193.3**
Platelet estimates (10^3/μL)						
Day 23	1,404.9 ± 50.8	1,213.3 ± 43.4	1,433.3 ± 62.0	1,369.7 ± 61.6	1,365.0 ± 46.1	1,449.0 ± 69.8
Week 14	1,022.7 ± 58.1	994.0 ± 55.0	997.5 ± 52.5	942.9 ± 42.5	858.9 ± 43.5	1,033.7 ± 79.9

TABLE 3
Selected Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Clinical Chemistry						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	10	10	10	10	10
Cholesterol (mg/dL)						
Day 5	106 ± 3	104 ± 4	98 ± 3	103 ± 3	104 ± 3	95 ± 4
Day 23	87 ± 2	86 ± 2	79 ± 6	82 ± 4*	78 ± 1**	78 ± 3**
Week 14	95 ± 2	111 ± 4	94 ± 2	87 ± 2	83 ± 2*	79 ± 2**
Triglycerides (mg/dL)						
Day 5	110 ± 12	115 ± 8	102 ± 9	86 ± 6	99 ± 8	116 ± 14
Day 23	94 ± 12	89 ± 7	95 ± 10	85 ± 11	63 ± 5	78 ± 8
Week 14	139 ± 18	116 ± 10	98 ± 9	81 ± 4**	76 ± 7**	59 ± 6**
Alanine aminotransferase (IU/L)						
Day 5	43 ± 2	56 ± 2**	70 ± 3**	81 ± 1**	80 ± 2**	81 ± 3**
Day 23	33 ± 1	48 ± 4**	65 ± 4**	74 ± 4**	71 ± 2**	74 ± 3**
Week 14	64 ± 5	437 ± 68**	218 ± 27**	245 ± 30**	246 ± 37**	248 ± 22**
Creatine kinase (IU/L)						
Day 5	370 ± 36	442 ± 50	458 ± 28	536 ± 54*	540 ± 49*	544 ± 52*
Day 23	397 ± 45	436 ± 56	638 ± 110	530 ± 72	561 ± 70	549 ± 105
Week 14	197 ± 23	311 ± 94	265 ± 23	296 ± 24**	359 ± 23**	432 ± 48**
Sorbitol dehydrogenase (IU/L)						
Day 5	23 ± 1	20 ± 1	20 ± 1	19 ± 1	19 ± 2	19 ± 1
Day 23	19 ± 1	18 ± 1	16 ± 1	14 ± 1**	13 ± 1**	15 ± 1**
Week 14	22 ± 2	101 ± 17**	65 ± 10**	81 ± 13**	96 ± 20**	103 ± 12**
Bile acids (µmol/L)						
Day 5	21.5 ± 1.7	20.2 ± 1.6	21.0 ± 1.4	24.3 ± 1.8	21.8 ± 1.8	20.0 ± 1.4
Day 23	22.9 ± 1.7	23.4 ± 1.9	19.9 ± 1.6	27.5 ± 1.5	27.5 ± 1.5	26.9 ± 4.0
Week 14	19.7 ± 2.5	50.4 ± 6.0**	39.9 ± 4.3**	35.3 ± 3.5	45.3 ± 5.6**	38.7 ± 3.2*

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

The small erythrocytes and erythrocyte fragments may have been erroneously classified as platelets resulting in the very high platelet counts observed throughout the study. However, a platelet estimate performed on blood smears on day 23 and at week 14 suggested an increased platelet count existed in exposed male rats on day 23, but no increased platelet counts occurred in exposed animals at week 14. The increased platelet counts on day 23 may indicate or be consistent with a general increase in hematopoiesis or possibly an iron deficiency-like process. Increased platelet counts have been demonstrated in instances of iron deficiency or iron deficiency-like processes (e.g., cupric sulfate administration; NTP, 1993).

Increased neutrophil and monocyte counts (primarily an effect at higher exposures) were considered to represent an inflammatory response related to the inflammatory lesions observed histologically (e.g., gastric lesions). Leukocyte and lymphocyte counts were increased. While the increases in neutrophil and monocyte counts probably contributed to the increased leukocyte counts, the apparent increases in lymphocyte counts appeared to be the controlling factor. The increases in lymphocyte counts were not consistent between sexes until week 14, when the increased lymphocyte counts were primarily an effect of high exposure and could suggest altered lymphocyte distribution peripherally.

Progressive increases in alanine aminotransferase and sorbitol dehydrogenase activities occurred in all exposed rats; on day 5, only alanine aminotransferase demonstrated the effect. By week 14, alanine aminotransferase activities were increased in all exposed groups by approximately 2- to 8-fold in males and 3- to 7-fold in females; sorbitol dehydrogenase activities were increased in all exposed groups by approximately 2- to 6-fold in males and 3- to 5-fold in females. These increases, however, did not occur in an exposure concentration-related fashion. Increased serum activities of alanine aminotransferase and sorbitol dehydrogenase suggest increased hepatocellular membrane leakage or injury.

Increased bile acid concentrations occurred on day 23 and progressed; by week 14, bile acid concentrations were increased in the 500 and 1,000 mg/L males and in most female groups. As with alanine aminotransferase and sorbitol dehydrogenase, these increases did not occur in an exposure concentration-related fashion. Increased bile acid concentration is typically used as a marker of cholestasis, but it may also occur in situations of hepatocellular injury or altered hepatic function. In this study, alkaline phosphatase and 5′-nucleotidase activity (serum enzyme markers of cholestasis) were decreased or unchanged. Thus, it would appear that bile acid concentration increases were related to a hepatocellular effect rather than a cholestatic event.

There was an apparent alteration in lipid metabolism, evidenced by decreases in cholesterol and triglyceride concentrations that appeared to affect males more than females. Small (approximately 8%) decreases in cholesterol concentration occurred on day 5 in all exposed males and progressed; by week 14, cholesterol concentrations were

decreased in 250, 500, and 1,000 mg/L males and 500 and 1,000 mg/L females. No exposure concentration-relationship was evident. Decreased triglyceride concentrations occurred on day 23 in males; by week 14, triglyceride concentrations were decreased in 1,000 mg/L males and 250, 500, and 1,000 mg/L females. An exposure concentration-related decrease was apparent in females. The mechanism of the the decreased serum lipids was unknown, but the cholesterol and triglyceride concentrations decreased by 20% and 42%, respectively, in 1,000 mg/L males and by 17% and 58%, respectively, in 1,000 mg/L females, at week 14.

Increased creatine kinase activities occurred on day 5 in 500 and 1,000 mg/L males and in 250, 500, and 1,000 mg/L females. By week 14, creatine kinase activities were increased in 250, 500, and 1,000 mg/L rats; the increases in 1,000 mg/L males and females were 75% and 120%, respectively. An exposure concentration-relationship was evident and suggests muscle injury.

In urine (Table C1), decreased volume and increased specific gravity were consistent with the observed decreases in water intake and suggested poor water palatability. The minor increases in urea nitrogen concentration were also consistent with decreased water intake and minimal dehydration. Transient, small ($\leq$6%) decreases in calcium concentration occurred on day 5 in exposed males and females. On day 23, transient, small ($\leq$12%) increases in phosphorus concentration that were unrelated to exposure concentration occurred in the 500 and 1,000 mg/L groups. The mechanism of these transient calcium and phosphorus changes was unknown. Changes in other clinical pathology variables were minor or sporadic.

Organ weight data are presented in Tables 4 and D1. Absolute and relative liver weights of males in the 500 and 1,000 mg/L groups were significantly less than those of the controls. Absolute spleen weights of 500 and 1,000 mg/L males and relative spleen weights of 250 and 500 mg/L males were also significantly less than those of the controls. Relative spleen and kidney weights of 500 and 1,000 mg/L females were significantly increased. Other differences in organ weights were considered to be related to the lower body weights of animals in these groups, rather than to a specific toxic effect of sodium dichromate dihydrate.

The administration of sodium dichromate dihydrate in the the drinking water of rats was associated with increased incidences of nonneoplastic lesions in the glandular stomach, duodenum, and pancreatic lymph nodes of males and females and in the liver and bone marrow of females (Tables 5, A1, and A2). The severities of the lesions in the duodenum, glandular stomach, and pancreatic lymph node were generally greater at the 1,000 mg/L exposure concentration.

TABLE 4
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	330 ± 8	322 ± 4	337 ± 6	330 ± 4	316 ± 3	298 ± 5**
Liver						
Absolute	10.89 ± 0.42	10.30 ± 0.28	11.45 ± 0.38	10.51 ± 0.18	9.20 ± 0.17**	8.88 ± 0.18**
Relative	32.91 ± 0.65	31.91 ± 0.61	33.98 ± 0.75	31.90 ± 0.54	29.15 ± 0.53**	29.80 ± 0.35**
Spleen						
Absolute	0.64 ± 0.02	0.60 ± 0.01	0.62 ± 0.02	0.60 ± 0.02	0.53 ± 0.01**	0.60 ± 0.01**
Relative	1.94 ± 0.03	1.85 ± 0.03	1.83 ± 0.04	1.81 ± 0.05*	1.69 ± 0.02**	2.00 ± 0.03
Female						
Necropsy body wt	193 ± 3	215 ± 3	199 ± 2	196 ± 2	193 ± 3	185 ± 2
R. Kidney						
Absolute	0.64 ± 0.02	0.71 ± 0.01*	0.71 ± 0.01	0.71 ± 0.02	0.69 ± 0.03	0.67 ± 0.02
Relative	3.34 ± 0.09	3.32 ± 0.04	3.55 ± 0.05	3.55 ± 0.07	3.58 ± 0.10*	3.63 ± 0.09*
Spleen						
Absolute	0.41 ± 0.01	0.44 ± 0.01	0.43 ± 0.01	0.44 ± 0.01	0.44 ± 0.01	0.44 ± 0.00
Relative	2.12 ± 0.05	2.04 ± 0.03	2.16 ± 0.05	2.22 ± 0.03	2.25 ± 0.05*	2.39 ± 0.03**

* 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). Statistical tests were performed on unrounded data.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
Intestine Small, Duodenum[a]	10	10	10	10	10	10
Infiltration Cellular, Histiocyte[b]	0	0	7**(1.1)[c]	9**(1.2)	8**(1.4)	7**(1.4)
Lymph Node, Pancreatic	10	10	10	10	10	10
Ectasia	0	0	0	0	1 (1.0)	10**(1.7)
Hyperplasia, Lymphoid	0	0	0	3 (1.0)	3 (1.0)	6**(2.7)
Infiltration, Cellular, Histiocyte	0	5* (1.0)	2 (1.0)	4* (1.0)	5* (1.0)	9**(1.8)
Stomach, Glandular	10	10	10	10	10	10
Ulcer	0	0	0	0	1 (2.0)	8**(3.0)
Epithelium, Hyperplasia, Focal, Regenerative	0	0	0	0	0	10**(2.2)
Epithelium, Metaplasia, Focal, Squamous	0	0	0	0	0	7**(2.6)
Female						
Intestine Small, Duodenum	10	10	10	10	10	10
Infiltration Cellular, Histiocyte	0	1 (1.0)	5* (1.0)	7**(1.4)	8**(1.6)	10**(1.7)
Liver	10	10	10	10	10	10
Infiltration Cellular, Histiocyte	0	3 (1.3)	6**(1.0)	6**(1.0)	9**(1.2)	8**(1.0)
Inflammation, Chronic, Focal	3 (1.0)	5 (1.0)	2 (1.0)	7 (1.0)	2 (1.0)	10**(1.0)
Lymph Node, Pancreatic	10	10	10	10	10	10
Ectasia	0	0	0	0	1 (1.0)	10**(1.8)
Hyperplasia, Lymphoid	0	0	2 (1.5)	0	0	10**(2.1)
Infiltration, Cellular, Histiocyte	4 (1.0)	8 (1.4)	7 (1.7)	7 (1.3)	7 (1.7)	9* (1.9)
Stomach, Glandular	10	10	10	10	10	10
Ulcer	0	0	0	0	0	10**(3.5)
Epithelium, Hyperplasia, Focal, Regenerative	0	0	0	0	0	10**(2.0)
Epithelium, Metaplasia, Focal, Squamous	0	0	0	0	0	10**(2.4)
Bone Marrow	10	10	10	10	10	10
Hyperplasia	0	0	0	0	0	4* (1.0)

* 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

In the glandular stomach, gross lesions described as deformity, pale foci, pale nodules, or thick, pale mucosa were observed in males and females exposed to 1,000 mg/L and correlated well with the microscopic lesions observed in this group. The lesions occurred immediately adjacent to the limiting ridge, the anatomic demarcation between the rodent forestomach and glandular stomach. Microscopically, the incidences of glandular stomach lesions, which included ulcers, regenerative epithelial hyperplasia, and squamous epithelial metaplasia were significantly increased in male and female rats exposed to 1,000 mg/L. These microscopic lesions were similar in all affected rats and were strikingly site specific within the glandular stomach, consistently occurring immediately adjacent to the limiting ridge. Ulcers were focal to focally extensive lesions characterized by complete loss of the lining of the mucosal epithelium with necrosis of the underlying tissue (Plates 2 and 3). Necrosis often extended through the submucosa and muscle layers. Invariably, mild to marked chronic inflammation consisting of infiltrates of neutrophils, macrophages, lymphocytes, and eosinophils in varying numbers and proliferation of fibrous connective tissue extended from the base of the ulcer through the submucosa to the serosal surface. Regenerative glandular hyperplasia occurred at the lateral borders of the ulcers as focal areas of irregular disorganized hyperplastic gastric glands lined by well-differentiated tall columnar epithelium (Plates 3, 4, and 5). Squamous epithelial metaplasia was diagnosed when well-differentiated, keratinized, squamous epithelium extended from the limiting ridge to partially or completely cover the ulcerated areas replacing the normal tall columnar epithelium of the gastric glands (Plates 4 and 5).

In the pancreatic lymph nodes, the incidences of minimal to mild histiocytic cell infiltration were increased in all exposed males and females; the increases were statistically significant in 1,000 mg/L females and in all exposed males, except the 125 mg/L group. The incidences of lymphoid hyperplasia and sinusoidal ectasia were significantly increased in 1,000 mg/L males and females. Histiocytic cell infiltrates were multifocal, randomly scattered, small clusters of enlarged macrophages with pale foamy cytoplasm (Plates 6 and 7). Lymphoid hyperplasia consisted of minimal to mild proliferation of lymphocytes, primarily in the paracortical areas, and sinusoid ectasia was characterized by minimal to mild dilatation of the subcapsular or medullary sinuses.

In the duodenum, the incidences of minimal to mild histiocytic infiltration were significantly increased in the groups exposed to 125 mg/L or greater. Histiocytic infiltrates occurred in the lamina propria at the tips of duodenal villi and were morphologically similar to those observed in the pancreatic lymph nodes (Plates 8 and 9).

In the liver, the incidences of minimal histiocytic cellular inflammation were significantly increased in 125 mg/L or greater females; focal chronic inflammation was significantly increased at 1,000 mg/L. Histiocytic infiltrates were randomly scattered and morphologically similar to those observed in the duodenum and pancreatic lymph nodes. Chronic inflammation consisted of scattered, small clusters of lymphocytes and macrophages occasionally mixed with a few neutrophils.

In the bone marrow, the incidence of minimal hyperplasia was significantly increased in 1,000 mg/L females.

3-Month Study in Male and Female B6C3F$_1$ Mice

All mice survived to the end of the study (Table 6). Final mean body weights and body weight gains of mice exposed to 125 mg/L or greater and the body weight gains of 62.5 mg/L male mice were significantly less than those of the control groups (Table 6 and Figure 2). Male and female mice exposed to 125 (except males at week 13), 250, 500, or 1,000 mg/L consumed less water than did the respective control groups. Exposure concentrations of 62.5, 125, 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 9, 15, 26, 45, and 80 mg/kg to mice. No clinical findings were attributed to sodium dichromate dihydrate exposure.

Table 6
Survival, Body Weights, and Water Consumption of Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	20.4 ± 0.4	37.9 ± 1.1	17.5 ± 0.9		4.3	3.8
62.5	10/10	21.3 ± 0.4	35.6 ± 0.8	14.3 ± 0.7**	94	4.4	4.5
125	10/10	21.6 ± 0.3	34.2 ± 1.2**	12.6 ± 1.2**	90	3.0	4.2
250	10/10	21.1 ± 0.6	32.6 ± 0.7**	11.5 ± 0.7**	86	3.0	3.3
500	10/10	20.7 ± 0.5	30.5 ± 0.9**	9.8 ± 0.8**	81	2.3	3.2
1,000	10/10	21.4 ± 0.4	30.5 ± 0.6**	9.1 ± 0.5**	80	2.4	2.8
Female							
0	10/10	17.9 ± 0.4	27.7 ± 0.7	9.8 ± 0.8		3.0	3.3
62.5	10/10	18.7 ± 0.3	28.0 ± 0.9	9.3 ± 0.7	101	3.1	2.9
125	10/10	18.7 ± 0.4	25.5 ± 0.6*	6.8 ± 0.4**	92	2.0	2.6
250	10/10	18.3 ± 0.4	25.5 ± 0.5*	7.3 ± 0.4**	92	2.1	2.4
500	10/10	18.1 ± 0.4	24.8 ± 0.3**	6.7 ± 0.3**	89	1.5	2.0
1,000	10/10	18.8 ± 0.3	24.1 ± 0.4**	5.3 ± 0.3**	87	1.1	1.5

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

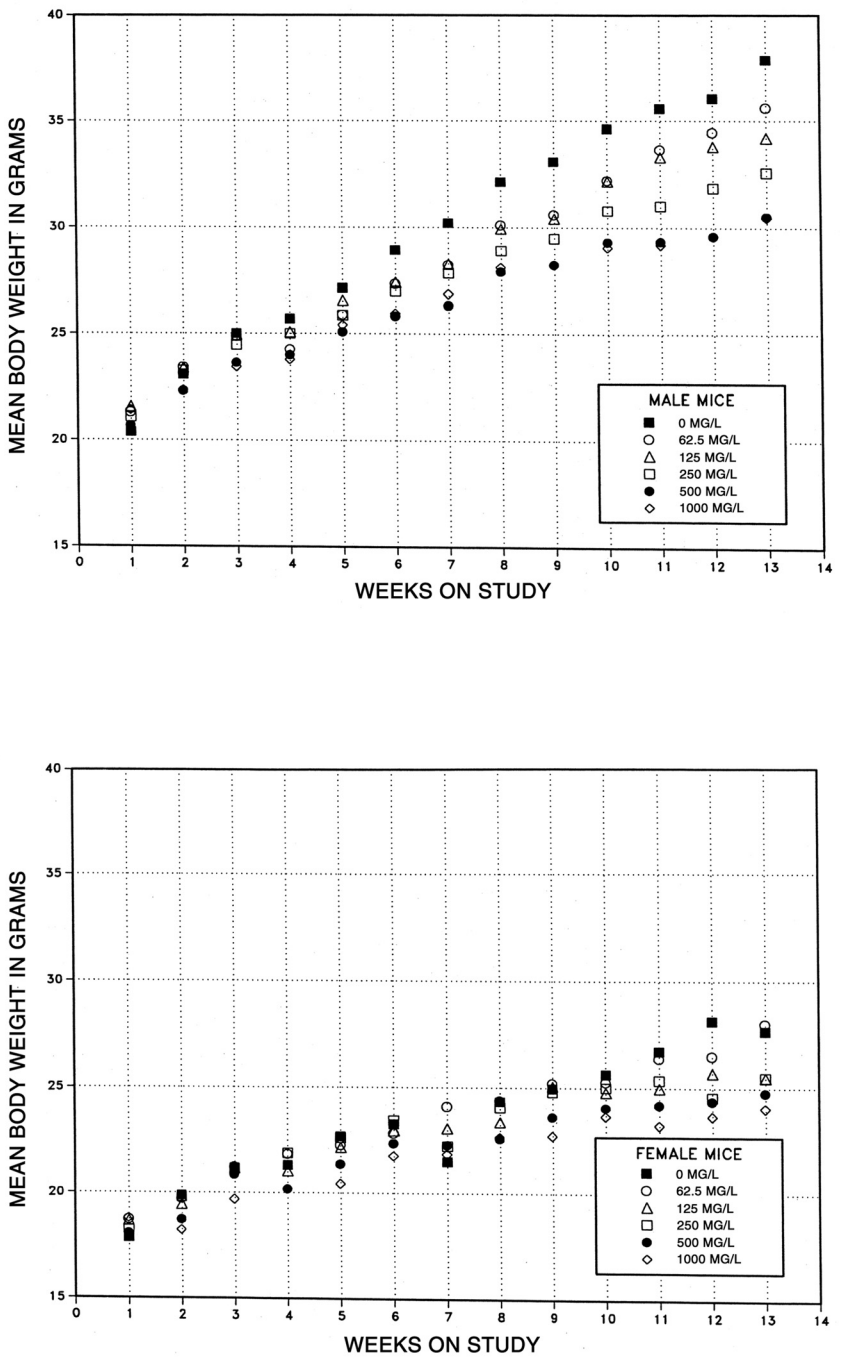


FIGURE 2
Body Weights of Mice Administered Sodium Dichromate Dihydrate in Drinking Water for 3 Months

The hematology data for B6C3F$_1$ mice in study 1 are listed in Table C2. The mice demonstrated an erythrocyte microcytosis (decrease in mean cell volume) similar to that seen in rats. However, the mice were much less affected, and no contradictory data from hematocrit values or mean cell hemoglobin concentrations, as described for rats, occurred for mice. The decreases in mean cell hemoglobin reflected the mean cell volume decrease. Similar to the occurrence at week 14 in the rat studies, erythrocyte counts increased, and hemoglobin concentrations decreased, but only in females.

Absolute liver weights of males exposed to 250 mg/L or greater and females exposed to 500 or 1,000 mg/L were significantly less than those of the respective controls, but liver weights relative to body weights were unchanged (Tables 7 and D2). Relative kidney weights of males exposed to 1,000 mg/L were significantly greater than those of the control group. Other differences in organ weights were attributed to the reduced body weights of the mice.

TABLE 7
Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.9 ± 1.1	36.9 ± 0.9	35.6 ± 1.0*	33.3 ± 0.8**	31.1 ± 1.1**	31.2 ± 0.5**
R. Kidney						
Absolute	0.28 ± 0.01	0.28 ± 0.01	0.26 ± 0.01	0.26 ± 0.01*	0.24 ± 0.01**	0.26 ± 0.01**
Relative	7.25 ± 0.11	7.68 ± 0.29	7.43 ± 0.35	7.75 ± 0.20	7.76 ± 0.30	8.18 ± 0.07**
Liver						
Absolute	1.60 ± 0.08	1.54 ± 0.05	1.50 ± 0.05	1.40 ± 0.05*	1.33 ± 0.06**	1.34 ± 0.04**
Relative	40.93 ± 1.22	42.01 ± 1.68	41.86 ± 1.00	42.35 ± 1.58	42.78 ± 1.31	42.94 ± 0.91
Female						
Necropsy body wt	28.2 ± 0.7	28.6 ± 0.8	26.3 ± 0.7	27.1 ± 0.8	25.1 ± 0.3**	24.8 ± 0.4**
Liver						
Absolute	1.15 ± 0.03	1.14 ± 0.04	1.06 ± 0.02	1.11 ± 0.04	1.04 ± 0.02*	0.99 ± 0.02**
Relative	40.96 ± 0.98	39.75 ± 0.71	40.32 ± 0.88	41.03 ± 0.74	41.54 ± 0.71	40.01 ± 0.78

* 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). Statistical tests were performed on unrounded data.

In the duodenum, the incidences of minimal to mild epithelial hyperplasia were significantly increased in all exposed groups, and severities increased slightly with increasing exposure concentration (Tables 8, A3, and A4). Compared to the controls, the duodenal villi were short, thick, and blunted, the crypts elongated, and diffuse hyperplasia of the crypt epithelium extended towards the tips of the villi (Plates 10 and 11). The hyperplasic epithelial cells were tall, columnar, densely packed, and stained more basophilically than the shorter columnar epithelial cells lining the duodenal villi of the control mice (Plates 12 and 13). There were also increased numbers of mitotic figures in the hyperplastic epithelium. In addition, the epithelial cells lining the tips of the villi of many of the exposed mice were swollen and had vacuolated cytoplasm. Collectively, these duodenal lesions suggest regenerative hyperplasia secondary to previous epithelial cell damage or degeneration. In mice receiving 125 mg/L or greater, the incidences of minimal to mild histiocytic cell infiltration in the duodenum and mesenteric lymph nodes (except 500 mg/L males) were significantly increased. Histiocytic cell infiltration in the duodenum and the mesenteric lymph nodes was morphologically similar to that observed in the duodenum and pancreatic lymph nodes of rats. Slight glycogen depletion in hepatocytes was noted in exposed groups, but because it was associated with poor weight gain or diminished food intake, it was not recorded as a lesion.

TABLE 8
Incidences of Selected Nonneoplastic Lesions in B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
Intestine Small, Duodenum[a]	10	10	10	10	10	10
Epithelium, Hyperplasia[b]	0	4* (1.0)[c]	5* (1.0)	10** (1.3)	10** (1.7)	10** (1.9)
Infiltration Cellular, Histiocyte	0	0	8** (1.3)	10** (1.8)	10** (2.1)	10** (1.8)
Lymph Node, Mesenteric	10	9	9	8	8	10
Infiltration, Cellular, Histiocyte	0	0	4* (1.0)	6** (1.0)	3 (2.0)	8** (1.3)
Female						
Intestine Small, Duodenum	10	10	9	10	10	10
Epithelium, Hyperplasia	0	7** (1.0)	8** (1.3)	10** (1.3)	10** (1.4)	10** (1.7)
Infiltration Cellular, Histiocyte	0	0	9** (1.1)	10** (1.1)	10** (1.5)	10** (1.4)
Lymph Node, Mesenteric	10	10	10	10	9	10
Infiltration Cellular, Histiocyte	0	0	6** (1.0)	6** (1.0)	4* (1.3)	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

STUDY 2
3-MONTH COMPARATIVE TOXICITY STUDY IN MALE B6C3F$_1$ MICE

All mice survived to the end of the study (Table 9). Statistically significant decreases in final mean body weights and body weight gains occurred in the 125 and 250 mg/L groups (Table 9 and Figure 3). Water consumption by the 250 mg/L group was less than that by the control group. Exposure concentrations of 62.5, 125, and 250 mg/L resulted in average daily doses of approximately 8, 15, and 26 mg/kg, respectively. No clinical findings of toxicity were observed.

TABLE 9
Survival, Body Weights, and Water Consumption of Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
0	10/10	24.3 ± 0.3	45.5 ± 0.8	21.2 ± 0.7		4.5	3.6
62.5	10/10	24.1 ± 0.3	45.1 ± 0.7	21.1 ± 0.7	99	4.3	3.9
125	10/10	24.3 ± 0.3	41.4 ± 1.0**	17.0 ± 1.0**	91	3.7	3.5
250	10/10	24.2 ± 0.2	35.4 ± 0.4**	11.1 ± 0.6**	88	3.0	2.8

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

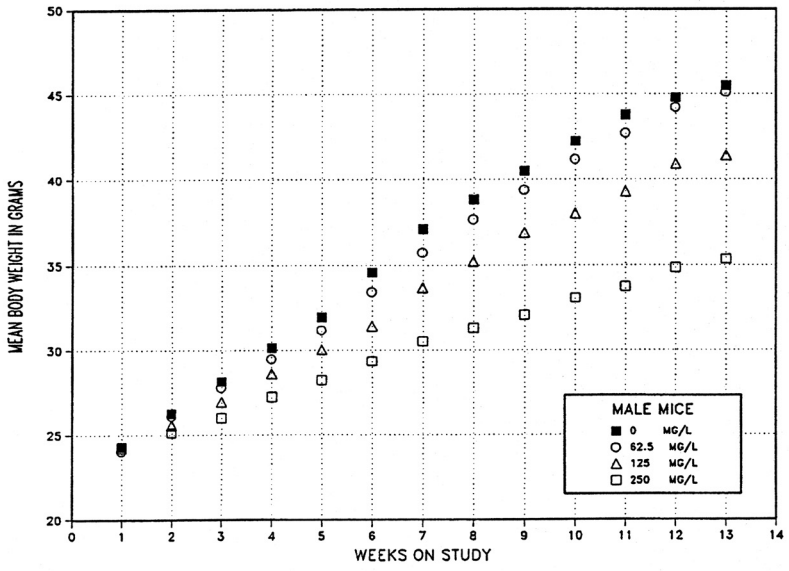


FIGURE 3
Body Weights of Male B6C3F$_1$ Mice Administered Sodium Dichromate Dihydrate in Drinking Water for 3 Months

Statistically significant decreases in absolute weight were seen in the kidney of the 125 mg/L group and in the kidney, lung, spleen, and thymus of the 250 mg/L group (Tables 10 and D3). Changes in organ weights were attributed to changes in body weights with the exception of thymus weight changes, which were considered related to treatment or stress.

Hematology and clinical chemistry data are summarized in Table C3. An erythrocyte microcytosis (decrease in mean cell volume) and a decrease in mean cell hemoglobin similar to those in the $B6C3F_1$ mice in study 1 and in the BALB/c and *am3*-C57BL/6 mice in study 2 occurred. The erythrocyte count in the 250 mg/L group was significantly increased.

Reproductive tissue evaluations in $B6C3F_1$ mice (Table E1) found no significant differences from controls in left cauda epididymis, left epididymis, or left testis weights or in spermatid measurements or epididymal sperm motility for any of the exposed groups.

TABLE 10
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male $B6C3F_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	10	10	10	10
Necropsy body wt	46.8 ± 0.8	46.7 ± 0.6	42.7 ± 1.0**	36.4 ± 0.5**
R. Kidney				
Absolute	0.33 ± 0.01	0.32 ± 0.01	0.30 ± 0.01*	0.26 ± 0.00**
Relative	6.94 ± 0.20	6.74 ± 0.16	7.01 ± 0.22	7.23 ± 0.10
Lung				
Absolute	0.22 ± 0.01	0.20 ± 0.01	0.20 ± 0.01	0.19 ± 0.00**
Relative	4.62 ± 0.14	4.35 ± 0.18	4.64 ± 0.22	5.13 ± 0.13
Spleen				
Absolute	0.09 ± 0.00	0.08 ± 0.00	0.08 ± 0.00	0.07 ± 0.00**
Relative	1.80 ± 0.06	1.69 ± 0.05	1.83 ± 0.08	1.87 ± 0.05
Thymus				
Absolute	0.07 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.05 ± 0.00*
Relative	1.42 ± 0.08	1.28 ± 0.13	1.41 ± 0.11	1.33 ± 0.08

* 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). Statistical tests were performed on unrounded data.

Incidences of minimal to mild histiocytic cellular infiltrates and mucosal epithelial hyperplasia in the duodenum of all exposed groups were significantly greater than those in the control groups, and, in general, the severities increased with increasing exposure concentration (Tables 11 and A5). The histiocytic infiltrates and epithelial hyperplasia were similar to those observed in the $B6C3F_1$ mice in study 1 (Plate 14). Incidences of minimal to mild glycogen depletion in the liver and minimal secretory depletion in the pancreas were significantly increased in the 125 and 250 mg/L groups. The severity of liver glycogen depletion in all exposed groups was greater than that of the controls.

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Male $B6C3F_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Intestine Small, Duodenum[a]	10	10	10	10
Infiltration Cellular, Histiocyte[b]	0	8**(1.0)[c]	10**(1.4)	10**(2.0)
Epithelium, Hyperplasia	0	4* (1.0)	10**(1.1)	10**(1.6)
Liver	10	10	10	10
Depletion Glycogen	1 (1.0)	2 (1.5)	9**(1.4)	10**(2.2)
Pancreas	10	10	10	10
Depletion, Secretory	0	2 (1.0)	7**(1.0)	9**(1.0)

* 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

3-Month Comparative Toxicity Study in Male BALB/c Mice

All mice survived to the end of the study (Table 12). Final mean body weights and body weight gains in the 125 and 250 mg/L groups were significantly less than those of the controls (Table 12 and Figure 4). Water consumption by the 250 mg/L group was less than that by the control group. Exposure concentrations of 62.5, 125, and 250 mg/L resulted in average daily doses of approximately 9, 14, and 24 mg/kg. Ruffled fur observed in the 250 mg/L group was attributed to reduced water consumption and decreased body weight gain.

Hematology and clinical chemistry data are summarized in Table C4. An erythrocyte microcytosis (decrease in mean cell volume) and a decrease in mean cell hemoglobin similar to those in the $B6C3F_1$ mice in study 1 and in the $B6C3F_1$ and *am3*-C57BL/6 mice in study 2 occurred. Increased erythrocyte counts occurred in the 125 and 250 mg/L groups. A small (30%) increase in alanine aminotransferase activity occurred in the 250 mg/L group, and total protein and albumin concentrations decreased (less than 12%) in the 125 and 250 mg/L groups.

Statistically significant decreases in absolute weights of the heart and kidney in 125 mg/L mice and of the heart, kidney, and liver in 250 mg/L mice were consistent with the mild reductions in body weights in the 125 and 250 mg/L groups (Table D4).

Reproductive tissue evaluations in male BALB/c mice are presented in Table E2. No differences in left cauda epididymis, left epididymis, or left testis weights or in spermatid or spermatozoal measurements were attributed to exposure to sodium dichromate dihydrate.

Table 12
Survival, Body Weights, and Water Consumption of Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
0	10/10	21.1 ± 0.3	28.2 ± 0.4	7.2 ± 0.3		3.4	3.2
62.5	10/10	21.2 ± 0.3	27.7 ± 0.5	6.6 ± 0.4	98	3.8	2.9
125	10/10	21.0 ± 0.4	26.3 ± 0.3**	5.3 ± 0.1**	93	2.9	2.5
250	10/10	21.1 ± 0.2	25.1 ± 0.4**	4.0 ± 0.3**	89	2.1	2.0

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

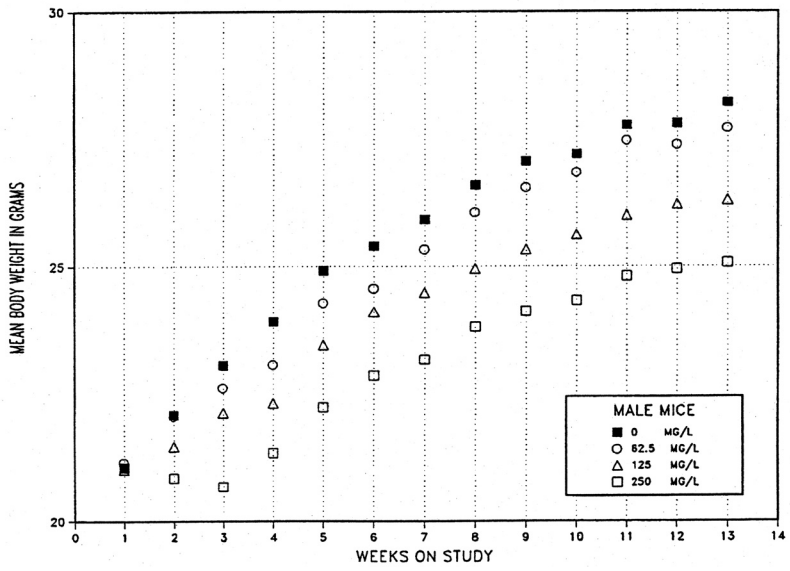


FIGURE 4
Body Weights of Male BALB/c Mice Administered Sodium Dichromate Dihydrate in Drinking Water for 3 Months

Except for epithelial hyperplasia in the 62.5 mg/L group, the incidences of histiocytic cellular infiltration and epithelial hyperplasia of the small intestine (duodenum) and secretory depletion of the pancreas were significantly increased in all exposed groups (Tables 13 and A6). Although, generally minimal to mild, there were slight increases in the average severities of these lesions with increasing exposure concentration. The histiocytic infiltrates and epithelial hyperplasia were similar to those observed in B6C3F$_1$ mice.

TABLE 13
Incidences of Selected Nonneoplastic Lesions in Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Intestine Small, Duodenum[a]	10	10	10	10
Infiltration Cellular, Histiocyte[b]	0	4* (1.0)[c]	9**(1.8)	10**(1.7)
Epithelium, Hyperplasia	0	2 (1.0)	10**(1.1)	10**(1.4)
Pancreas	10	10	10	10
Depletion, Secretory	0	6**(1.0)	9**(1.3)	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 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

3-Month Comparative Toxicity Study in Male *am3*-C57BL/6 Mice

All core study mice survived to the end of the study (Table 14). Final mean body weights and body weight gains of all exposed groups were significantly less than those of the control group (Table 14 and Figure 5). Water consumption by 250 mg/L mice was less than that by the controls. Exposure concentrations of 62.5, 125, and 250 mg/L resulted in average daily doses of approximately 8, 15, and 25 mg/kg, respectively. No clinical findings of toxicity were observed in core study mice exposed to sodium dichromate dihydrate.

Hematology and clinical chemistry data are summarized in Table C5. An erythrocyte microcytosis (decrease in mean cell volume) and a decrease in mean cell hemoglobin similar to those in the $B6C3F_1$ mice in study 1 and in the $B6C3F_1$ and BALB/c mice in study 2 occurred. Decreases occurred in automated hematocrit values in the 125 and 250 mg/L groups and in the manual hematocrit value and hemoglobin concentration in the 250 mg/L group. An increase (92%) in alanine aminotransferase activity occurred in the 250 mg/L group.

Statistically significant decreases in absolute heart, liver, and thymus weights and absolute and relative spleen weights in the 250 mg/L core study group were related to decreased body weights (Table D5).

Table 14
Survival, Body Weights, and Water Consumption of Core Study Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
0	5/5	25.3 ± 0.5	41.3 ± 1.5	16.0 ± 1.7		3.5	3.3
62.5	5/5	24.9 ± 0.8	35.4 ± 2.4*	10.5 ± 1.9*	86	3.3	3.5
125	5/5	25.1 ± 0.4	34.3 ± 1.3**	9.3 ± 0.9**	83	5.9	3.3
250	5/5	25.0 ± 0.7	27.2 ± 0.3**	2.2 ± 0.5**	66	2.5	2.4

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 3 months/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

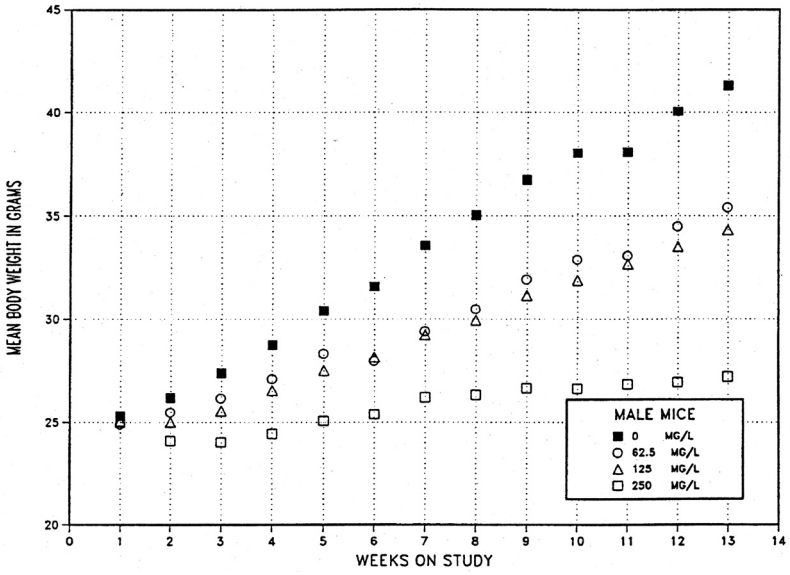


FIGURE 5
Body Weights of Core Study Male *am3*-C57BL/6 Mice Administered Sodium Dichromate Dihydrate in Drinking Water for 3 Months

Reproductive tissue evaluations in male *am3*-C57BL/6 mice are presented in Table E3. A statistically significant decrease in the left testis weight observed in the 250 mg/L group was related to the decreased body weight in this group. No other significant differences in reproductive parameters were observed.

In the duodenum of core study mice, incidences of histiocytic cellular infiltration at 125 and 250 mg/L and epithelial hyperplasia at 62.5 mg/L and greater were significantly greater than those in the controls, and severities of these lesions increased with exposure concentration (Tables 15 and A7). Incidences of glycogen depletion in the liver of all exposed groups, secretory depletion in the pancreas of the 125 and 250 mg/L groups, and histiocytic cellular infiltration of the mesenteric lymph node in the 250 mg/L group were also significantly increased compared to those in the control group. The histiocytic infiltrates and epithelial hyperplasia were similar to those observed in B6C3F_1 mice.

TABLE 15
Incidences of Selected Nonneoplastic Lesions in Core Study Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Intestine Small, Duodenum[a]	5	5	5	5
Infiltration Cellular, Histiocyte[b]	0	2 (1.0)[c]	5**(1.4)	4* (1.8)
Epithelium, Hyperplasia	0	5**(1.0)	5**(1.2)	5**(1.8)
Liver	5	5	5	5
Depletion Glycogen	0	4* (2.0)	5**(1.6)	5**(3.8)
Pancreas	5	5	5	5
Depletion, Secretory	0	3 (1.0)	4* (1.0)	5**(1.6)
Lymph Node, Mesenteric	5	5	5	5
Infiltration Cellular, Histiocyte	0	0	0	4* (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 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

GENETIC TOXICOLOGY

Sodium dichromate dihydrate (5 to 300 µg/plate) was mutagenic in *Salmonella typhimurium* strains TA100 and TA98 and in *Escherichia coli* strain WP2 uvrA pKM101 with and without 10% induced rat liver S9 enzymes (Table B1). Responses were stronger in the strains that mutate via base substitution (TA100, *E. coli* WP2); in all three tester strains, mutagenicity was more pronounced in the absence of S9, based on the lowest concentration that elicited a significant mutagenic response.

The results of four micronucleus tests conducted in three strains of mice were mixed. In study 1, micronucleus frequencies were determined in peripheral blood erythrocytes of male and female B6C3F_1 mice administered sodium dichromate dihydrate over an exposure concentration range of 62.5 to 1,000 mg/L for 3 months. No significant increases were seen in micronucleated normochromatic erythrocytes in male or female mice over the exposure concentration range tested; there was a decrease in the percentage of polychromatic erythrocytes among total erythrocytes (an indication of bone marrow toxicity), but the changes were small and not well correlated with exposure concentrations (Table B2).

In study 2, micronucleus frequencies were evaluated in male B6C3F_1, BALB/c, and *am3*-C57BL/6 mice administered sodium dichromate dihydrate over an exposure concentration range of 62.5 to 250 mg/L in drinking water for 3 months. An increase in micronucleated erythrocytes that was judged to be equivocal was noted in male B6C3F_1 mice (Table B3), based on the trend test (P=0.031), which showed an increase in micronucleated normochromatic erythrocytes that did not reach statistical significance (required P value of 0.025); no exposed groups were significantly increased over the control group in this study. No increase in micronucleated normochromatic erythrocytes was observed in male BALB/c mice (Table B4). A significant exposure concentration-related increase (P<0.001) in micronucleated erythrocytes was noted in male *am3*-C57BL/6 mice (Table B5). In this study, two of three dose groups were significantly (P<0.008) elevated over the control group. No significant effect of chemical exposure on the percentage of polychromatic erythrocytes was observed in any of the three micronucleus tests conducted in study 2.

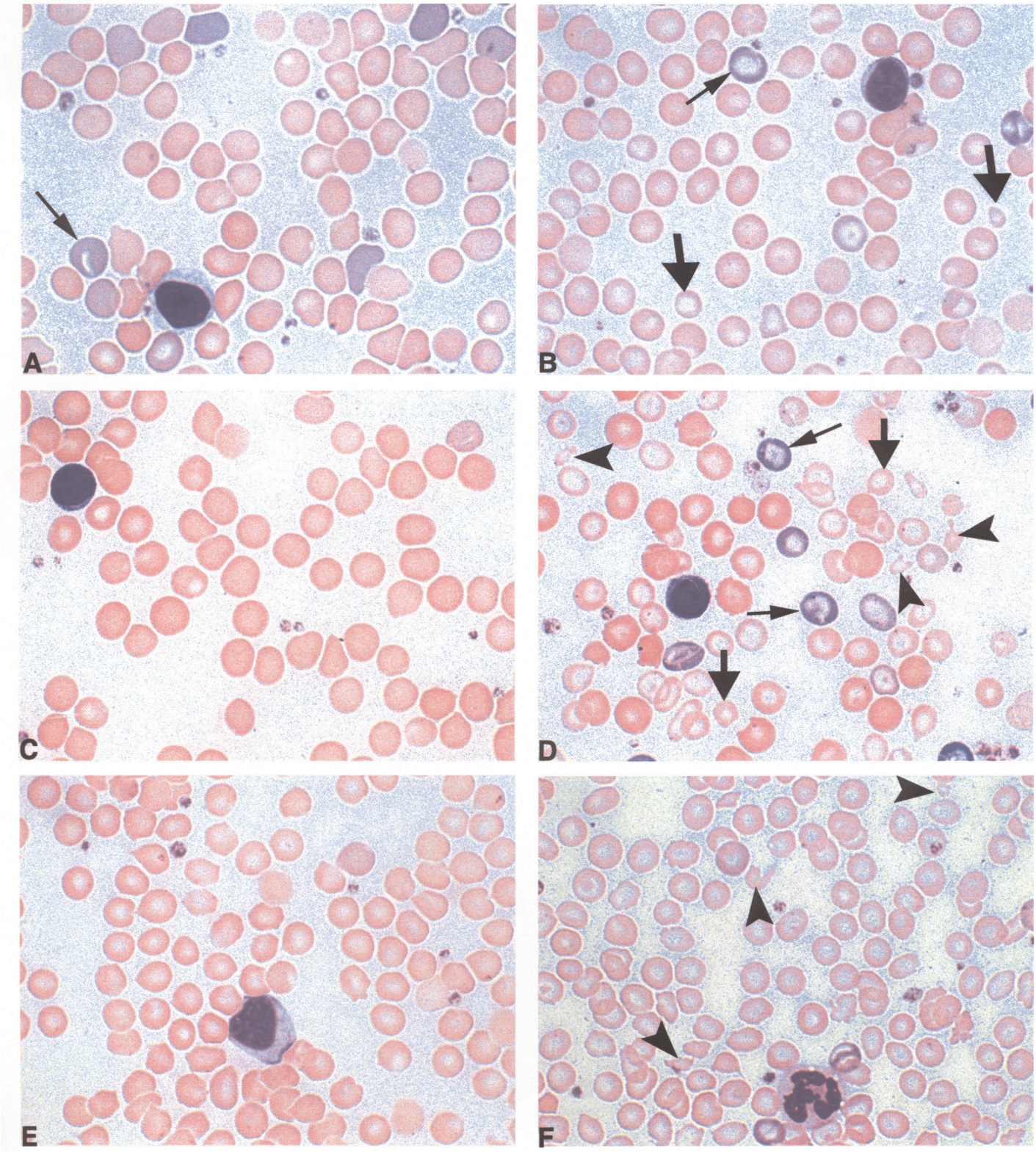


Plate 1

Blood film of control (A, C, E) and 1,000 mg/L (B, D, F) rats in the 3-month study of sodium dichromate dihydrate. At day 5 (A and B), immature erythrocytes (thin arrows) were observed in all exposure groups and were considered an age-related finding. In the 1,000 mg/L rats, there was a slight increase in the overall central pallor of the erythrocytes and microcytes (thick arrows). At day 21 (C and D), the incidence of immature erythrocytes decreased in the controls but was increased in the 1,000 mg/L rats (thin arrows). The increased central pallor of erythrocytes was more pronounced (hypochromatophilic erythrocytes), and there were increased incidences of microcytes (thick arrows) and erythrocyte fragments (arrowheads). At the end of the study (E and F), the incidences of immature erythrocytes ameliorated in the 1,000 mg/L rats and were similar to those in the controls. An increase in erythrocyte central pallor was still evident, but the pallor was not as severe as on day 21. Erythrocyte fragments were still present in increased numbers (arrowheads).

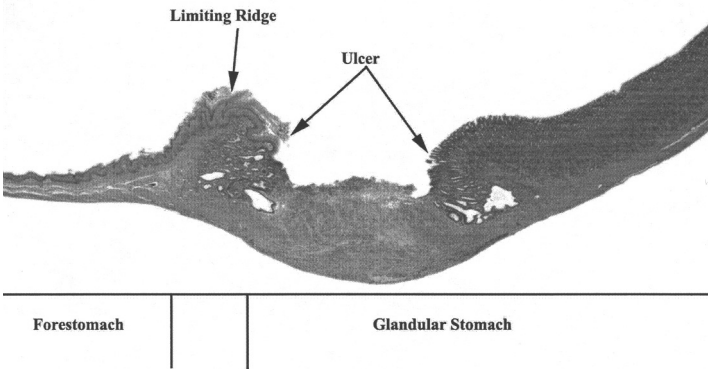


PLATE 2
Low magnification showing the anatomy of the rodent stomach and the location of focal ulceration in the glandular stomach immediately adjacent to the demarcation (limiting ridge) between the glandular stomach and forestomach. Male rat exposed to 1,000 mg/L sodium dichromate dihydrate for 3 months. H&E; 2.5×

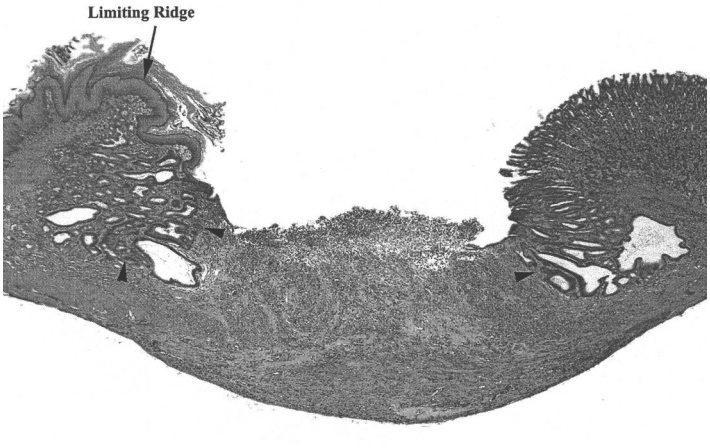


PLATE 3
Higher magnification of Plate 2. The ulcer is characterized by focal loss of the mucosal epithelium with associated intense chronic inflammation and fibrosis extending through the submucosa and muscle layers. Note focal areas of glandular epithelial hyperplasia at the margins of the ulcer (arrowheads). H&E; 10×

PLATE 4
Squamous epithelial metaplasia (arrows) in the glandular stomach of a male rat exposed to 1,000 mg/L sodium dichromate dihydrate for 3 months. Squamous epithelium similar to that covering the forestomach and limiting ridge has replaced the normal mucosal glandular epithelium. H&E; 10×

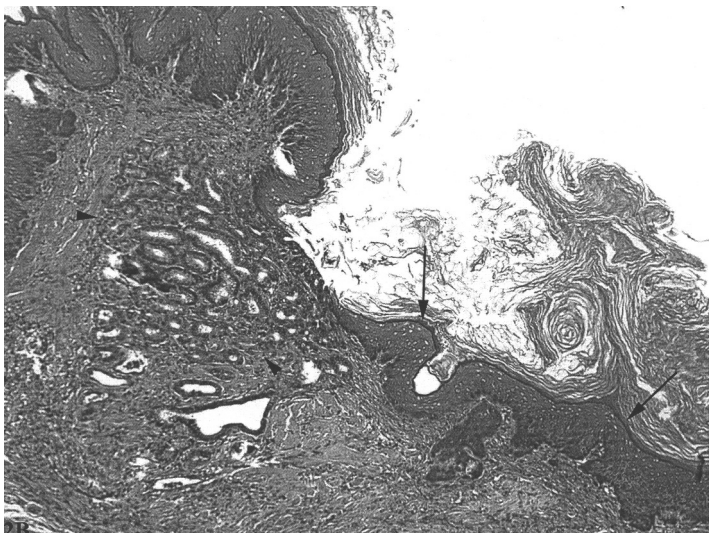


PLATE 5
Higher magnification of Plate 4 showing metaplastic squamous epithelium (arrowheads). Note hyperplasia glandular epithelium at the margin of the ulcer and chronic inflammation and fibrosis in the submucosal tissue (arrows). H&E; 20×

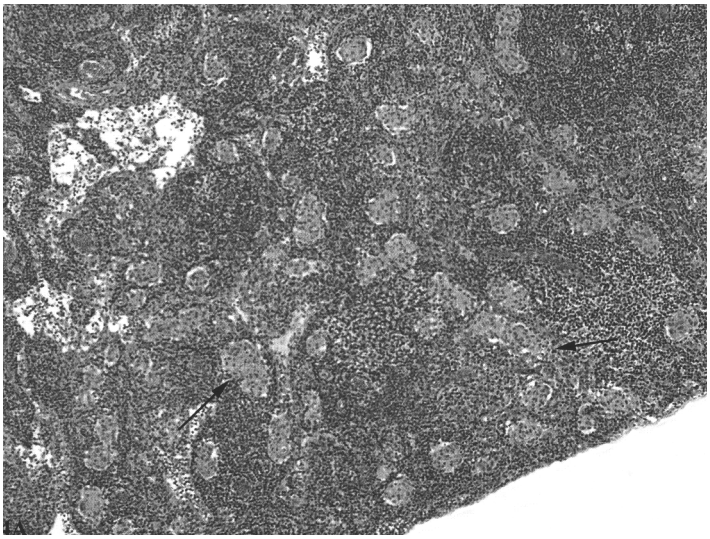


PLATE 6
Pancreatic lymph node of a male rat exposed to 1,000 mg/L sodium dichromate dihydrate for 3 months showing multiple clusters of histiocytic infiltrates (arrows). H&E; 5×

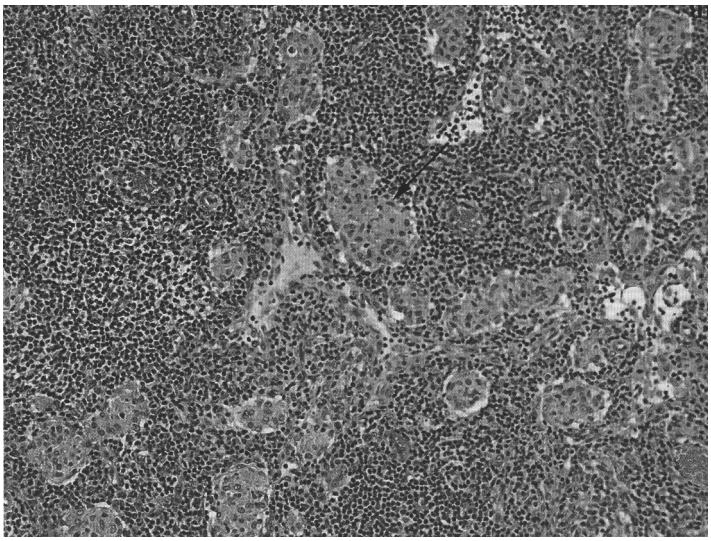


PLATE 7
Higher magnification of Plate 6 showing clusters of pale macrophages (histiocytes) (arrow). H&E; 20×

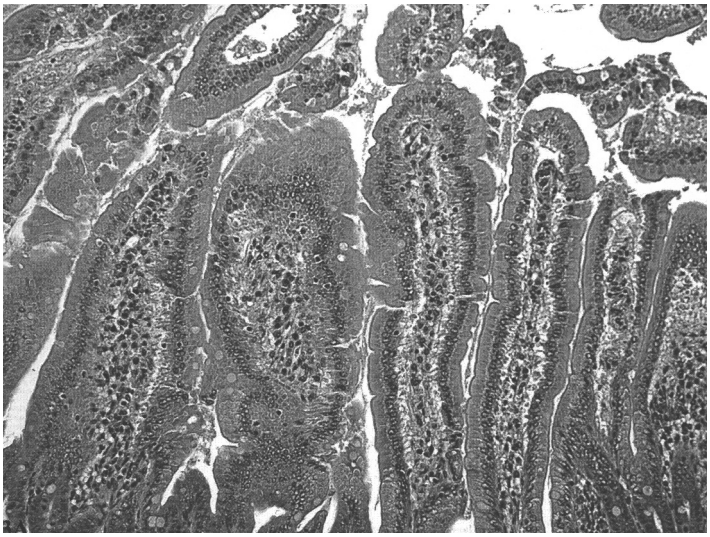


PLATE 8
Normal microscopic anatomy of the duodenum of a control rat from the 3-month study showing elongate mucosal villi. H&E; 20×

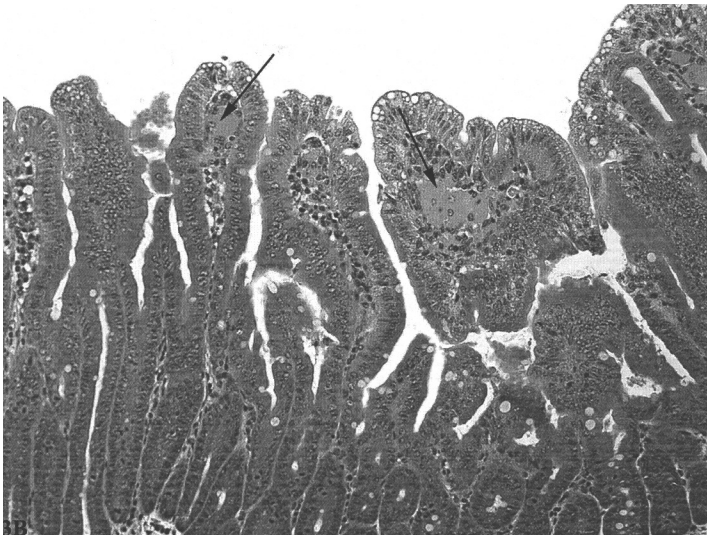


PLATE 9
Duodenum of a male rat exposed to 1,000 mg/L sodium dichromate dihydrate for 3 months showing focal clusters of pale macrophages (histiocytes) in the lamina propria (arrows). H&E; 20×

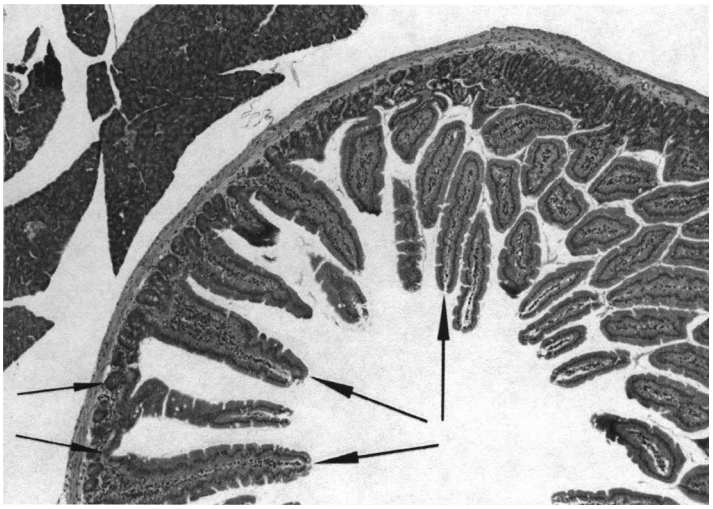


PLATE 10
Duodenum from a male control mouse in the 3-month study of sodium dichromate dihydrate illustrating normal microscopic anatomy. Note tall, slender villi (long arrows) and short crypts (short arrows). H&E; 10×

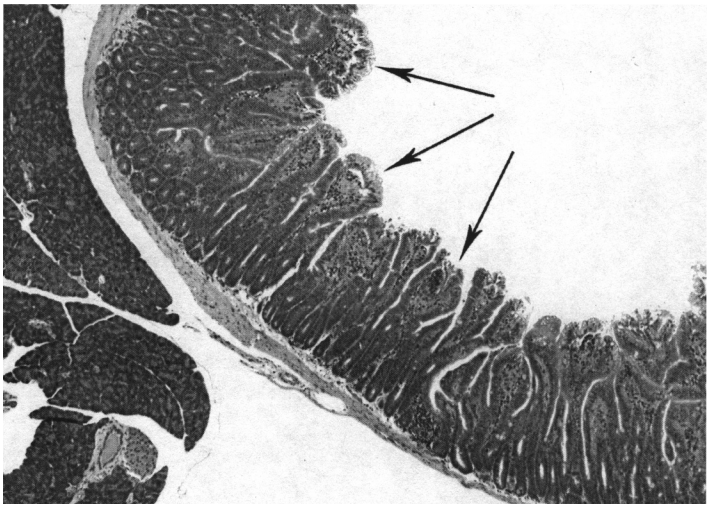


PLATE 11
Duodenum from a male B6C3F$_1$ mouse exposed to 1,000 mg/L sodium dichromate dihydrate for 3 months. Duodenal villi (arrows) are short, blunt, and wide, and crypts are elongate. H&E; 10×

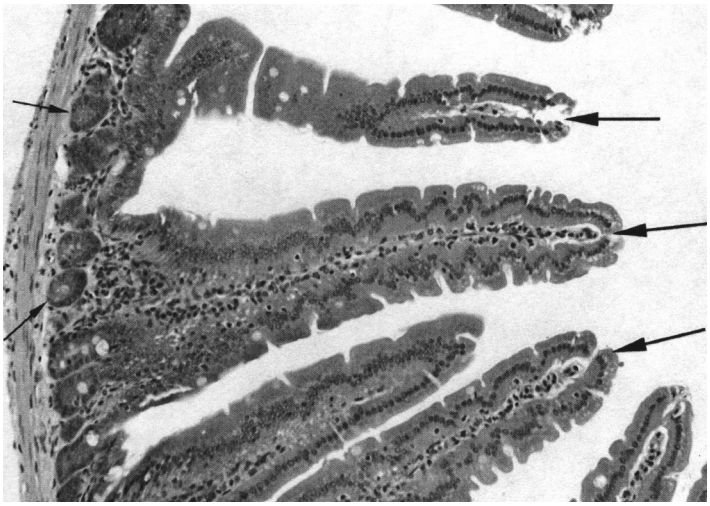


PLATE 12
Higher magnification of Plate 10. Duodenal villi (arrows) are tall and slender with tapered tips and lined by tall columnar epithelial cells, and crypts are short. H&E; 25×

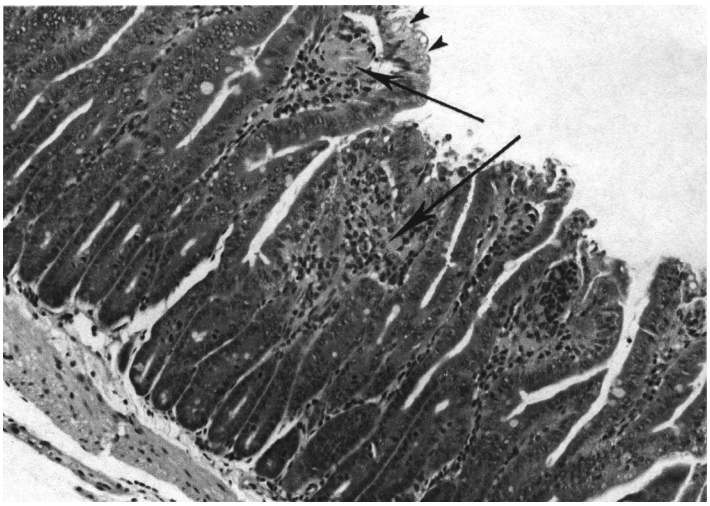


PLATE 13
Higher magnification of Plate 11. Note the hyperplastic crypt epithelium extending along short, wide villi and the epithelium piling up in some areas. Also note the swollen vacuolated epithelial cells at the tip of one villus (arrowheads) and histiocytic infiltrates within the lamina propria (arrows). H&E; 25×

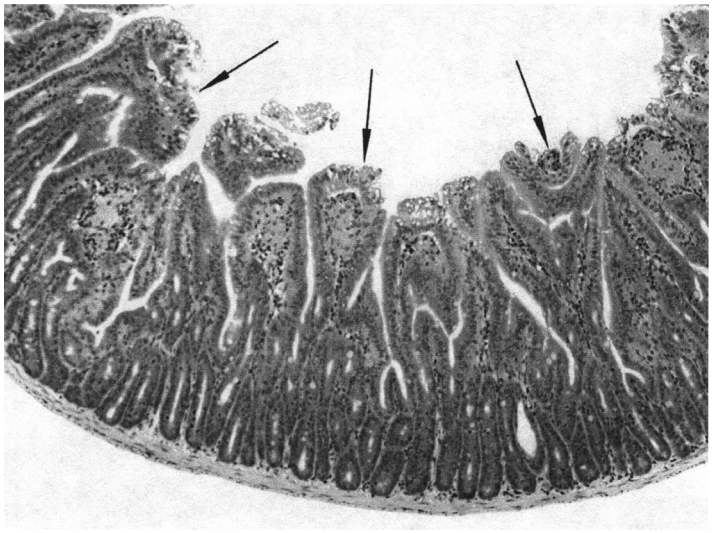


PLATE 14

Hyperplasia of the duodenal epithelium from a male B6C3F$_1$ mouse exposed to 250 mg/L sodium dichromate dihydrate for 3 months in the comparative study. Note the short villi (arrows) and elongate crypts. Also note significant piling up of the crypt epithelium and extension of the hyperplastic epithelium along the villi. H&E; 25×

DISCUSSION

The studies in this report were performed primarily to aid in the design and dose selection for 2-year carcinogenicity studies of sodium dichromate dihydrate (hexavalent chromium, Cr VI) administered in drinking water. Preliminary toxicokinetic studies in mice, rats, and guinea pigs were performed to determine whether the apparent kinetics of absorption of chromium differed significantly between rodents that possess a forestomach (rats and mice), and that are typically used in cancer studies, with animals not possessing a forestomach, in this case guinea pigs (Appendix G). Because it was proposed that the acidic environment of the glandular stomach would promote reduction of Cr VI to trivalent chromium (Cr III), and because Cr III was thought to be both less well absorbed and relatively nontoxic (Proctor *et al.*, 2002), it was important to determine whether the presence of a relatively nonacidic forestomach might afford enhanced chromium absorption. The guinea pig was considered a suitable animal to test this theory because, in addition to lacking a forestomach, this species requires vitamin C in the diet, providing further reducing capacity in the gut. The results of these studies indicated that the presence of a forestomach did not materially affect chromium absorption kinetics. While differences were seen in the patterns of tissue chromium accumulation among the three species, the results for chromium concentrations in blood and kidneys in rats and mice were in general agreement with expectations based on values reported in the literature. The chromium concentrations in the blood of guinea pigs suggested somewhat greater absorption than did the concentrations in the blood of the rats or mice.

The drinking water concentrations of sodium dichromate dihydrate used in these studies ranged up to 1,000 mg/L in the initial 3-month studies with male and female F344/N rats and B6C3F$_1$ mice. A lower top concentration of 250 mg/L was used in the comparative toxicity studies in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice because reduced body weight gain and water consumption were seen at the higher exposure concentrations in the initial studies. However, 250 mg/L produced reductions in body weight gain in all three strains that were greater than expected based on the first 3-month study in B6C3F$_1$ mice. The decreases in water consumption observed in rats and mice were likely due to poor palatability of the drinking solutions containing sodium dichromate dihydrate. The marked changes observed in body weights and the minor changes in organ-weight-to-body-weight ratios were considered more likely related to the reduced water consumption than to toxicity of sodium dichromate dihydrate. In male and female rats exposed to 1,000 mg/L, the reduction in body weight was considered related to a combination of chemical-induced stomach ulcers and reduced water consumption.

The decreases in urine volume and increases in urine specific gravity observed in male and female rats receiving sodium dichromate dihydrate were likely due to reduced water consumption and the poor palatability of the dosed water. Oliguria and proteinuria observed in rats receiving 100 mg potassium chromate/kg body weight per day for 28 days in drinking water (Diaz-Mayans *et al.*, 1986) were also likely due to reduced water consumption. Histological evaluation of the kidneys of rats receiving similar doses of Cr VI from sodium dichromate dihydrate in this study did not reveal morphological changes.

Sodium dichromate dihydrate administration in the drinking water resulted in a generally exposure concentration-related microcytic hypochromic anemia in rats. Microcytosis was evidenced by decreased mean cell volume and by microcytic changes (erythrocyte fragments, keratocytes, and blebbing) observed microscopically. Anemia was evidenced by decreased hematocrit and hemoglobin. Microcytic hypochromic anemia was also observed in mice as indicated by decreases in mean cell volume and mean cell hemoglobin, but the changes were much less severe in mice and occurred only at the higher exposures. In contrast, in rats, significant changes in hematologic indices consistent with a responsive anemia were observed at all exposures (as low as 1.9 mg chromate/kg body weight per day). Decreases in mean cell volume and mean cell hemoglobin were previously seen in rats and mice given potassium chromate in feed for 9 weeks (NTP 1996a,b). No-observed-adverse-effect levels for mean cell volume and mean cell hemoglobin changes were reported as 2.1 or 2.45 mg chromium/kg body weight per day in male and female rats and 7.35 or 12 mg/kg for male and female mice. These numbers are very consistent with the present findings. Studies in which rats were exposed to Cr III as 3.6 mg potassium chromate/kg body weight in drinking water for 1 year resulted in no changes in routine hematology (MacKenzie *et al.*, 1958). In 13-week studies, no significant hematologic or clinical chemistry changes were observed in F344/N rats or B6C3F$_1$ mice fed diets containing chromium picolinate monohydrate at concentrations up to 50,000 ppm (resulting in exposure concentrations of up to 1,143 mg Cr III/kg body weight per day to rats and 3,204 or 2,463 mg/kg to male and female mice, respectively) (Rhodes *et al.*, 2005). These observations suggest that, at the exposure concentrations used in the current studies, Cr VI is absorbed and is sufficiently bioavailable to cause toxicity to the hematopoietic system.

Reduction of Cr VI to reactive intermediates may have resulted in damage to hemoglobin and cell membranes during hematopoiesis that functionally interferes with the cellular uptake of iron or association of iron with hemoglobin. The binding of chromium to erythrocyte hemoglobin may also have interfered with the utilization of iron by erythrocytes. Chromium is thought to attach to the erythrocyte membrane to be transported intracellularly by the general anion carrier, and then to bind to hemoglobin (Ottenwaelder *et al.*, 1988; Alexander and Aaseth, 1995). Cr VI is reduced in both the plasma and in erythrocytes to pentavalent chromium (Cr V) and quadravalent chromium (Cr IV), which upon autooxidation back to Cr VI can produce reactive oxygen species (superoxide anion, hydroxyl radicals) that can cause oxidative damage to hemoglobin and erythrocyte membrane lipids (ATSDR, 2000; Fernandes *et al.*, 2000).

The decreased reticulocyte response that was observed on day 5 and the relatively mild reticulocyte response that was observed on day 23 are consistent with an adverse compound-related effect on hematopoiesis. Cr VI is associated with generation of reactive DNA-damaging intermediates that could damage erythropoietic precursors; however, compound-associated interference with hemoglobin synthesis would also decrease a reticulocyte response. The majority of the erythrocyte morphology findings that were observed in this study, including erythrocyte fragments, poikilocytes, keratocytes, etc., are all potentially fragmentation changes that are consistent with changes observed in iron deficiency anemia and oxidative damage to erythrocyte membranes and hemoglobin. The anemia (decrease in spun hematocrit and hemoglobin) that was observed in rats in the sodium dichromate dihydrate-exposed groups in this study was of greater magnitude on day 23 than at week 14, and group mean erythrocyte counts were observed to be increased at week 14. The increases in erythrocyte counts that were observed at week 14 are consistent with an adaptive response to continued microcytosis and low cellular hemoglobin. This type of adaptive response is also observed in rats and humans with aluminum toxicity; aluminum, like chromium, is taken up by erythrocytes (Mahieu *et al.*, 2000) and appears to cause decreased intraerythrocytic ferritin, a marker of iron content, even in the presence of normal iron stores (Caramelo *et al.*, 1995).

Regarding other evidence for systemic or site of contact toxicity of exposure to sodium dichromate dihydrate, a significantly increased incidence of ulcer and epithelial hyperplasia and metaplasia of the glandular stomach occurred in rats in the 1,000 mg/L group. Coincident with the focal ulcers was evidence of inflammation within the stomach wall. The increases in blood neutrophil and monocyte counts were consistent with an inflammatory response. These lesions occurred at the junction between the forestomach and the glandular stomach, where the reduction of Cr VI to Cr V and to Cr IV and subsequent radical formation would be enhanced by the increased acidity.

Histiocytic infiltration was consistently noted as a minor lesion in the duodenum of the small intestine of rats and mice receiving sodium dichromate dihydrate. Hyperplasia, characterized by villi that were taller than normal and with tightly packed basophilic epithelial cells, was an additional finding in the small intestine in all strains of mice. Histiocytic infiltration was also seen in the pancreatic and mesenteric lymph nodes in rats and in B6C3F_1 and *am3*-C57BL/6 mice, but not in BALB/c mice. This type of small intestine lesion has been observed following exposure to a variety of other materials including certain hair dyes, pigments, lipids, and phospholipids (Gopinath *et al.*, 1987). These compounds are thought to initially induce infiltration of macrophages into the lamina propria of the small intestine and subsequently histiocytosis in the mesenteric lymph node, but the significance of the lesion is not known. The macrophages do not often show degenerative changes, and it is postulated that they are unable to degrade phagocytized materials. Curiously, histiocytic infiltration was also clearly noted in the livers of female rats in all exposed groups, but was not seen in exposed male rats or in exposed mice.

Liver injury after oral ingestion of Cr VI compounds has been infrequently reported. Hepatocyte cytoplasmic vacuolization was observed in mice in a 9-week feed study of potassium dichromate (NTP, 1996a). The highest dose of Cr VI used was 32 mg/kg per day, which is slightly higher than the amount of Cr VI consumed by rats in the 1,000 mg/L groups in the current study. Clinical pathology measures in the rat study gave some evidence of hepatocyte injury. Serum alanine aminotransferase activities were increased in all exposed groups of male and female rats, but sorbitol dehydrogenase activities were much less affected. In contrast, the only other liver lesion reported in rats was chronic focal inflammation in females. However, this lesion occurred in controls as well as exposed rats and did not show a strong exposure concentration relationship.

The sporadic increases in serum bile acid concentrations in both sexes of rats receiving sodium dichromate dihydrate also suggest cholestasis. However, other markers of cholestasis, namely alkaline phosphatase and 5′-nucleotidase activities, were either decreased or remained unchanged. Accordingly, the increase in bile acid concentration was not considered a cholestatic event. Overall, the evidence for significant hepatotoxicity in rats was weak, at best.

The study to compare the toxic responses in three mouse strains was performed primarily to confirm and expand the findings reported by the NTP (1996a) concerning the observations of a liver effect (increased hepatocyte vacuolization) in a reproductive toxicity study in BALB/c mice. Therefore, the study was performed using B6C3F_1, BALB/c, and *am3*-C57BL/6 (considered relatively insensitive to many hepatotoxicants) strains. There was little difference in the response of the three strains to sodium dichromate dihydrate. All showed an exposure concentration-related decrease in water consumption and body weight gain, an increase in erythrocytic microcytosis, histiocytic infiltration of the small intestines, and pancreatic secretory depletion. However, there were differences in some clinical pathology parameters including serum alanine aminotransferase, which showed higher increases in exposed BALB/c mice compared to the B6C3F_1 and *am3*-C57BL/6 mice. In addition to the pancreatic secretory depletion, exposed B6C3F_1 and *am3*-C57BL/6 mice showed liver glycogen depletion that was likely also due to depressed food consumption, which is frequently observed when water consumption is decreased. Thus, with the exception of the minor alanine aminotransferase response, this study did not confirm the earlier findings with the BALB/c strain, and instead suggests that the liver effects in all three strains more likely reflected nutritional inequalities than a toxic response to sodium dichromate dihydrate.

Serum creatine kinase activity was increased in a significant positive trend, with the activity of this enzyme in the three highest exposure concentration groups of male and female rats being significantly greater than those of the controls. Increased creatine kinase activity is an indicator of muscle injury. The reasons for and the tissue origins accounting for these increases are not known.

In summary, administration of sodium dichromate dihydrate in the drinking water to F344/N rats and $B6C3F_1$ mice resulted in focal ulceration, hyperplasia, and metaplasia in the glandular stomach at the limiting ridge in rats in the 1,000 mg/L group and evidence of increased histiocytic infiltration in the liver (female), duodenum of the small intestine, and/or pancreatic lymph nodes at concentrations as low as 62.5 mg/L, the lowest concentration studied. In addition, a microcytic, hypochromic anemia occurred at all exposure concentrations and was considered evidence of a toxic response resulting from absorption of Cr VI following oral ingestion in rats. A similar, but less severe, anemia was evident in mice receiving drinking water containing sodium dichromate dihydrate; histiocytic infiltration was noted in the duodenum of all three strains studied ($B6C3F_1$, BALB/c, and *am3*-C57BL/6) at all concentrations employed, in the mesenteric lymph nodes at 125 mg/L or greater in the $B6C3F_1$ strain, and at 250 mg/L in the *am3*-C57BL/6 strain. There was no consistent evidence of hepatocyte injury in mice in any of the strains tested. Variations in glycogen content were considered more likely related to diminished food intake than to the toxicity of sodium dichromate dihydrate.

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APPENDIX A
SUMMARY OF NEOPLASMS AND NONNEOPLASTIC LESIONS IN RATS AND MICE

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TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
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						
Intestine small, duodenum	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte			7 (70%)	9 (90%)	8 (80%)	7 (70%)
Liver	(10)	(10)	(10)	(10)	(9)	(10)
Hematopoietic cell proliferation, focal	2 (20%)					
Hepatodiaphragmatic nodule					1 (11%)	
Infiltration cellular, mixed cell	2 (20%)	1 (10%)				1 (10%)
Inflammation, chronic, focal			2 (20%)		2 (22%)	1 (10%)
Centrilobular, vacuolization cytoplasmic	1 (10%)					
Pancreas	(10)	(6)	(6)	(6)	(6)	(10)
Acinus, atrophy, focal						2 (20%)
Salivary glands	(10)	(6)	(6)	(6)	(6)	(10)
Parotid gland, basophilic focus, multiple	7 (70%)	3 (50%)	4 (67%)	4 (67%)	3 (50%)	10 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Ulcer						1 (10%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Ulcer					1 (10%)	8 (80%)
Epithelium, hyperplasia, focal, regenerative						10 (100%)
Epithelium, metaplasia, focal, squamous						7 (70%)
Cardiovascular System						
Heart	(10)	(6)	(6)	(6)	(6)	(10)
Cardiomyopathy, focal	6 (60%)	5 (83%)	3 (50%)	5 (83%)	6 (100%)	4 (40%)
Endocrine System						
Adrenal cortex	(10)	(6)	(6)	(6)	(6)	(10)
Accessory adrenal cortical nodule	1 (10%)			1 (17%)		
Hyperplasia, focal				1 (17%)		
Hypertrophy, focal						1 (10%)
Pituitary gland	(10)	(6)	(6)	(6)	(6)	(10)
Pars distalis, angiectasis				1 (17%)		
Pars distalis, cyst	2 (20%)	1 (17%)	1 (17%)			
General Body System						
None						
Genital System						
Testes	(10)	(6)	(6)	(6)	(6)	(10)
Atrophy	1 (10%)				1 (17%)	

TABLE A1
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				2 (20%)		3 (30%)
Lymph node	(10)	(10)	(10)	(9)	(10)	(10)
Mediastinal, hemorrhage	2 (20%)					1 (10%)
Pancreatic, ectasia					1 (10%)	10 (100%)
Pancreatic, hyperplasia, lymphoid				3 (33%)	3 (30%)	6 (60%)
Pancreatic, infiltration, cellular, histiocyte		5 (50%)	2 (20%)	4 (44%)	5 (50%)	9 (90%)
Lymph node, mesenteric	(10)	(6)	(6)	(6)	(6)	(10)
Ectasia					1 (17%)	
Infiltration, cellular, histiocyte	1 (10%)	1 (17%)		3 (50%)		1 (10%)
Spleen	(10)	(6)	(6)	(6)	(6)	(10)
Hematopoietic cell proliferation						1 (10%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(6)	(6)	(6)	(6)	(10)
Inflammation, acute, focal						1 (10%)
Inflammation, chronic, focal	3 (30%)	1 (17%)	2 (33%)	2 (33%)	1 (17%)	3 (30%)
Metaplasia, osseous						1 (10%)
Alveolar epithelium, bronchiole, hyperplasia					1 (17%)	
Alveolus, infiltration cellular, histiocyte	1 (10%)					2 (20%)
Special Senses System						
Harderian gland	(10)	(6)	(6)	(6)	(6)	(10)
Inflammation, chronic, focal						2 (20%)
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Casts protein			1 (10%)		1 (10%)	
Nephropathy	5 (50%)	1 (10%)	1 (10%)	2 (20%)	4 (40%)	4 (40%)

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
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						
Intestine small, duodenum	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte		1 (10%)	5 (50%)	7 (70%)	8 (80%)	10 (100%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)			1 (10%)	1 (10%)	2 (20%)
Infiltration cellular, histiocyte		3 (30%)	6 (60%)	6 (60%)	9 (90%)	8 (80%)
Inflammation, chronic, focal	3 (30%)	5 (50%)	2 (20%)	7 (70%)	2 (20%)	10 (100%)
Necrosis, focal				1 (10%)	1 (10%)	
Salivary glands	(10)	(6)	(6)	(6)	(6)	(10)
Parotid gland, basophilic focus, multiple	6 (60%)	2 (33%)	2 (33%)	1 (17%)	2 (33%)	5 (50%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Muscularis, inflammation, chronic active, focal						1 (10%)
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Degeneration, cystic						1 (10%)
Ulcer						10 (100%)
Epithelium, hyperplasia, focal, regenerative						10 (100%)
Epithelium, metaplasia, focal, squamous						10 (100%)
Muscularis, inflammation, chronic active, focal						1 (10%)
Cardiovascular System						
Heart	(10)	(6)	(6)	(6)	(6)	(10)
Cardiomyopathy, focal	3 (30%)		2 (33%)	2 (33%)	1 (17%)	1 (10%)
Endocrine System						
Adrenal cortex	(10)	(6)	(6)	(6)	(6)	(9)
Accessory adrenal cortical nodule						1 (11%)
Pituitary gland	(10)	(6)	(6)	(6)	(6)	(10)
Rathke's cleft, cyst	1 (10%)		1 (17%)	1 (17%)	1 (17%)	4 (40%)
General Body System						
None						
Genital System						
Clitoral gland	(10)	(6)	(6)	(6)	(6)	(9)
Duct, ectasia	1 (10%)					
Ovary	(10)	(6)	(6)	(6)	(6)	(10)
Cyst	2 (20%)		1 (17%)			2 (20%)
Uterus	(10)	(6)	(6)	(6)	(6)	(10)
Hydrometra	2 (20%)		1 (17%)			1 (10%)

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Hematopoietic System						
Bone marrow	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia						4 (40%)
Lymph node	(10)	(10)	(10)	(10)	(10)	(10)
Mediastinal, hemorrhage	2 (20%)					1 (10%)
Mediastinal, hyperplasia, lymphoid	1 (10%)					
Pancreatic, ectasia					1 (10%)	10 (100%)
Pancreatic, hemorrhage	1 (10%)				1 (10%)	1 (10%
Pancreatic, hyperplasia, lymphoid			2 (20%)			10 (100%)
Pancreatic, infiltration, cellular, histiocyte	4 (40%)	8 (80%)	7 (70%)	7 (70%)	7 (70%)	9 (90%)
Lymph node, mesenteric	(10)	(6)	(6)	(6)	(6)	(10)
Infiltration, cellular, histiocyte	3 (30%)	2 (33%)	3 (50%)	2 (33%)	5 (83%)	6 (60%)
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(5)	(6)	(6)	(6)	(10)
Infiltration cellular, histiocyte				1 (17%)		
Inflammation, chronic, focal	4 (40%)	3 (60%)	1 (17%)		1 (17%)	2 (20%)
Metaplasia, osseous	1 (10%)					
Alveolus, infiltration cellular, histiocyte		1 (20%)				
Special Senses System						
Harderian gland	(10)	(6)	(6)	(6)	(6)	(10)
Infiltration cellular, lymphoid					1 (17%)	
Inflammation, chronic, focal			1 (17%)	1 (17%)		
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Casts protein				1 (10%)	1 (10%)	1 (10%)
Nephropathy						1 (10%)
Pelvis, inflammation			1 (10%)			

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
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						
Intestine small, duodenum	(10)	(10)	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte			8 (80%)	10 (100%)	10 (100%)	10 (100%)
Epithelium, hyperplasia		4 (40%)	5 (50%)	10 (100%)	10 (100%)	10 (100%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation, focal	1 (10%)	1 (10%)			2 (20%)	1 (10%)
Infiltration, cellular, focal, mixed cell	1 (10%)			1 (10%)	1 (10%)	1 (10%)
Necrosis, focal	2 (20%)			1 (10%)		
Pancreas	(10)	(6)	(6)	(5)	(6)	(10)
Cyst	1 (10%)					
Salivary glands	(10)	(6)	(6)	(6)	(5)	(10)
Submandibular gland, depletion secretory	4 (40%)	3 (50%)	3 (50%)	3 (50%)	2 (40%)	4 (40%)
Stomach, forestomach	(10)	(9)	(9)	(10)	(10)	(10)
Edema, focal	1 (10%)					3 (30%)
Stomach, glandular	(10)	(9)	(10)	(10)	(10)	(10)
Epithelium, dilatation, focal		2 (22%)				2 (20%)
Cardiovascular System						
None						
Endocrine System						
Thyroid gland	(10)	(6)	(6)	(6)	(6)	(10)
Follicle, degeneration, cystic		1 (17%)				
General Body System						
None						
Genital System						
Preputial gland	(10)	(6)	(6)	(6)	(6)	(10)
Atrophy	1 (10%)					
Duct, ectasia	1 (10%)					
Hematopoietic System						
Lymph node	(2)	(4)	(3)	(6)	(3)	(3)
Pancreatic, infiltration, cellular, histiocyte			2 (67%)	6 (100%)	3 (100%)	2 (67%)
Lymph node, mesenteric	(10)	(9)	(9)	(8)	(8)	(10)
Atrophy					1 (13%)	
Infiltration, cellular, histiocyte			4 (44%)	6 (75%)	3 (38%)	8 (80%)
Spleen	(10)	(6)	(6)	(6)	(6)	(10)
Hematopoietic cell proliferation		1 (17%)				
Pigmentation, focal, hemosiderin						1 (10%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Nephropathy		1 (10%)		2 (20%)	1 (10%)	2 (20%)
Renal tubule, vacuolization cytoplasmic	9 (90%)	10 (100%)	10 (100%)	9 (90%)	8 (80%)	2 (20%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
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						
Intestine small, duodenum	(10)	(10)	(9)	(10)	(10)	(10)
Infiltration cellular, histiocyte			9 (100%)	10 (100%)	10 (100%)	10 (100%)
Epithelium, hyperplasia		7 (70%)	8 (89%)	10 (100%)	10 (100%)	10 (100%)
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation, focal						4 (40%)
Infiltration cellular, focal, mixed cell	8 (80%)	2 (20%)	3 (30%)	4 (40%)	3 (30%)	4 (40%)
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Inflammation, acute, focal						1 (10%)
Ulcer	1 (10%)					
Epithelium, hyperplasia, focal		1 (10%)	1 (10%)			
Stomach, glandular	(10)	(10)	(10)	(10)	(10)	(10)
Epithelium, dilatation, focal	1 (10%)			1 (10%)		1 (10%)
Cardiovascular System						
None						
Endocrine System						
Adrenal cortex	(10)	(6)	(6)	(6)	(6)	(10)
Vacuolization cytoplasmic	10 (100%)	6 (100%)	6 (100%)	5 (83%)	2 (33%)	5 (50%)
Parathyroid gland	(10)	(6)	(6)	(6)	(6)	(10)
Cyst					1 (17%)	1 (10%)
General Body System						
None						
Genital System						
Uterus	(10)	(10)	(10)	(10)	(10)	(10)
Hypoplasia						1 (10%)
Endometrium, hyperplasia, cystic			1 (10%)			
Hematopoietic System						
Lymph node	(1)	(1)	(2)	(2)	(1)	(2)
Pancreatic, infiltration, cellular, histiocyte			1 (50%)	1 (50%)	1 (100%)	2 (100%)
Lymph node, mandibular	(10)	(6)	(6)	(6)	(6)	(10)
Hyperplasia, lymphoid	1 (10%)					
Lymph node, mesenteric	(10)	(10)	(10)	(10)	(9)	(10)
Infiltration, cellular, histiocyte			6 (60%)	6 (60%)	4 (44%)	9 (90%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)	(6)	(6)	(6)	(6)	(10)
Inflammation, chronic, focal				1 (17%)		
Special Senses System						
None						
Urinary System						
Kidney	(10)	(6)	(6)	(6)	(6)	(10)
Cyst	1 (10%)					
Nephropathy		1 (17%)				

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Survivors				
Terminal sacrifice	10	10	10	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine small, duodenum	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte		8 (80%)	10 (100%)	10 (100%)
Epithelium, hyperplasia		4 (40%)	10 (100%)	10 (100%)
Liver	(10)	(10)	(10)	(10)
Depletion glycogen	1 (10%)	2 (20%)	9 (90%)	10 (100%)
Hematopoietic cell proliferation	1 (10%)			
Inflammation	10 (100%)	7 (70%)	6 (60%)	5 (50%)
Necrosis, focal	3 (30%)		1 (10%)	
Pancreas	(10)	(10)	(10)	(10)
Depletion secretory		2 (20%)	7 (70%)	9 (90%)
Stomach, glandular	(10)	(10)	(10)	(10)
Erosion			1 (10%)	
Glands, vacuolization cytoplasmic		2 (20%)		
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
None				
Hematopoietic System				
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte			1 (10%)	3 (30%)
Integumentary System				
None				
Musculoskeletal System				
None				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Nervous System				
None				
Respiratory System				
None				
Special Senses System				
None				
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Renal tubule, regeneration	2 (20%)	1 (10%)	2 (20%)	3 (30%)
Renal tubule, vacuolization cytoplasmic	10 (100%)	10 (100%)	10 (100%)	9 (90%)

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

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Survivors				
Terminal sacrifice	10	10	10	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine small, duodenum	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte		4 (40%)	9 (90%)	10 (100%)
Epithelium, hyperplasia		2 (20%)	10 (100%)	10 (100%)
Liver	(10)	(10)	(10)	(10)
Fatty change, diffuse	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Hematopoietic cell proliferation				1 (10%)
Inflammation	3 (30%)	5 (50%)	2 (20%)	
Mineralization	1 (10%)			
Necrosis, focal	3 (30%)	2 (20%)		
Pancreas	(10)	(10)	(10)	(10)
Depletion secretory		6 (60%)	9 (90%)	10 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte		1 (10%)	1 (10%)	
Stomach, glandular	(10)	(10)	(10)	(10)
Inflammation				1 (10%)
Mineralization				1 (10%)
Cardiovascular System				
None				
Endocrine System				
None				
General Body System				
None				
Genital System				
Preputial gland	(10)			(10)
Abscess				1 (10%)
Testes	(10)			(10)
Germinal epithelium, degeneration	5 (50%)			8 (80%)
Hematopoietic System				
None				
Integumentary System				
None				

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Musculoskeletal System				
None				
Nervous System				
None				
Respiratory System				
Nose	(10)			(10)
Olfactory epithelium, atrophy, focal				1 (10%)
Special Senses System				
None				
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Infiltration cellular, mixed cell				1 (10%)
Medulla, renal tubule, mineralization		1 (10%)		
Renal tubule, casts	2 (20%)	1 (10%)		
Renal tubule, dilatation, focal		1 (10%)		
Renal tubule, regeneration	3 (30%)	6 (60%)	4 (40%)	3 (30%)

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

TABLE A7
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Core Study Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Disposition Summary				
Animals initially in study	5	5	5	5
Survivors				
Terminal sacrifice	5	5	5	5
Animals examined microscopically	5	5	5	5
Alimentary System				
Intestine small, duodenum	(5)	(5)	(5)	(5)
Infiltration cellular, histiocyte		2 (40%)	5 (100%)	4 (80%)
Epithelium, hyperplasia		5 (100%)	5 (100%)	5 (100%)
Liver	(5)	(5)	(5)	(5)
Depletion glycogen		4 (80%)	5 (100%)	5 (100%)
Hematopoietic cell proliferation	1 (20%)	1 (20%)	1 (20%)	1 (20%)
Infiltration cellular, mononuclear cell			1 (20%)	
Inflammation	5 (100%)	5 (100%)	5 (100%)	5 (100%)
Tension lipidosis	2 (40%)			
Pancreas	(5)	(5)	(5)	(5)
Depletion secretory		3 (60%)	4 (80%)	5 (100%)
Necrosis, focal	1 (20%)			
Cardiovascular System				
None				
Endocrine System				
Pituitary gland	(5)			(5)
Meningioma malignant, metastatic, brain				1 (20%)
General Body System				
None				
Genital System				
Epididymis	(5)			(5)
Inflammation, chronic				1 (20%)
Seminal vesicle	(5)		(1)	(5)
Dilatation			1 (100%)	
Testes	(5)			(5)
Germinal epithelium, degeneration				1 (20%)
Hematopoietic System				
Lymph node, mesenteric	(5)	(5)	(5)	(5)
Infiltration cellular, histiocyte				4 (80%)
Integumentary System				
None				

TABLE A7
Summary of the Incidence of Neoplasms and Nonneoplastic Lesions in Core Study Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Musculoskeletal System				
Bone	(5)			(5)
Maxilla, meningioma malignant, metastatic, brain				1 (20%)
Nervous System				
Brain	(5)			(5)
Meningioma malignant				1 (20%)
Respiratory System				
None				
Special Senses System				
Eye	(5)			(5)
Optic nerve, meningioma malignant, metastatic, brain				1 (20%)
Urinary System				
Kidney	(5)	(5)	(5)	(5)
Cyst			1 (20%)	
Hydronephrosis		1 (20%)		
Renal tubule, casts	1 (20%)			
Renal tubule, regeneration		1 (20%)		
Renal tubule, vacuolization cytoplasmic	4 (80%)	5 (100%)	2 (40%)	

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

APPENDIX B
GENETIC TOXICOLOGY

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TABLE B1
Mutagenicity of Sodium Dichromate Dihydrate in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	–S9 Trial 3	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+10% rat S9 Trial 3
TA100	0	41 ± 3.5	65 ± 9.2	56 ± 15.9	81 ± 13.5	71 ± 8.7	69 ± 12.6
	5	45 ± 2.7	106 ± 7.2	86 ± 4.8			
	10	55 ± 3.4	117 ± 17.6	115 ± 4.4	77 ± 6.5	79 ± 11.7	72 ± 11.6
	25	116 ± 6.4	272 ± 3.5	202 ± 23.1			
	50	151 ± 15.1	386 ± 22.2	202 ± 10.2	92 ± 7.8	120 ± 3.2	104 ± 3.2
	75	24 ± 2.6	154 ± 18.2	45 ± 20.3			
	100				331 ± 9.5	378 ± 43.8	316 ± 9.9
	200				91 ± 37.1	135 ± 8.6	368 ± 43.6
	300				5 ± 1.9	2 ± 0.9	36 ± 2.4
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		609 ± 28.8	459 ± 4.9	570 ± 13.0	1,121 ± 88.5	768 ± 36.1	670 ± 88.1
TA98	0	32 ± 2.5	21 ± 2.3	23 ± 3.0	19 ± 3.3	26 ± 2.5	27 ± 2.5
	5	40 ± 2.2	29 ± 1.2				
	10	52 ± 7.9	33 ± 2.1	58 ± 5.8	19 ± 3.8	26 ± 6.3	26 ± 2.1
	15		59 ± 5.9				
	20		55 ± 2.0				
	25	64 ± 6.5		37 ± 7.9	18 ± 3.4		29 ± 1.5
	30		12 ± 2.0				
	50	Toxic		Toxic	31 ± 0.9[d]	36 ± 2.5	29 ± 0.3
	75	Toxic		Toxic	43 ± 4.7[d]		65 ± 6.5
	100			Toxic	54 ± 4.7[d]	81 ± 3.8	90 ± 7.1
	150			Toxic			6 ± 1.5
	200			Toxic		Toxic	Toxic
	300					Toxic	
Trial summary		Positive	Positive	Negative	Positive	Positive	Positive
Positive control		413 ± 13.9	572 ± 8.4	471 ± 24.3	1,137 ± 18.8	840 ± 81.0	1,261 ± 29.6

	–S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
***Escherichia coli* WP2 uvrA pKM101 (Analogous to *S. typhimurium* TA102)**				
0	172 ± 7.1	208 ± 6.0	217 ± 17.2	254 ± 47.0
10	584 ± 56.6	531 ± 61.1	212 ± 7.0	260 ± 8.5
50	797 ± 10.0	601 ± 27.1	347 ± 44.2	403 ± 25.0
100	698 ± 19.1	547 ± 52.0	919 ± 39.2	963 ± 58.5
150	638 ± 9.7	589 ± 21.1	973 ± 25.0	915 ± 26.9
200	473 ± 56.0	365 ± 27.2	933 ± 27.0	962 ± 12.4
Trial summary	Positive	Positive	Positive	Positive
Positive control	853 ± 65.0	724 ± 12.3	821 ± 28.0	789 ± 19.3

[a] Study was performed at SITEK Research Laboratories. 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Precipitate on plate

TABLE B2
Frequency of Micronuclei in Normochromatic Erythrocytes in Peripheral Blood of Male and Female B6C3F$_1$ Mice Following Administration of Sodium Dichromate Dihydrate in Drinking Water for 3 Months[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[d] (%)
Male					
Water[e]	0	5	2.70 ± 0.46		4.1
Sodium dichromate dihydrate	62.5	5	2.60 ± 0.48	0.5547	3.5
	125	5	2.20 ± 0.51	0.7627	3.1
	250	5	3.70 ± 0.44	0.1053	3.3
	500	5	2.50 ± 0.42	0.6094	2.7
	1,000	5	2.00 ± 0.52	0.8467	3.3
			P=0.857[f]		
Female					
Water	0	5	1.70 ± 0.37		3.6
Sodium dichromate dihydrate	62.5	5	1.20 ± 0.34	0.8236	2.5
	125	5	1.60 ± 0.29	0.5692	3.4
	250	5	1.80 ± 0.30	0.4328	3.9
	500	5	2.10 ± 0.37	0.2580	3.2
	1,000	5	1.90 ± 0.24	0.3693	2.7
			P=0.158		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.005 (ILS, 1990)
[d] Percentage of polychromatic erythrocytes (reticulocytes) among total erythrocytes
[e] Untreated control
[f] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE B3
Frequency of Micronuclei in Normochromatic Erythrocytes in Peripheral Blood of Male B6C3F$_1$ Mice Following Administration of Sodium Dichromate Dihydrate in Drinking Water for 3 Months (Study 2)[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[d] (%)
Water[e]	0	5	2.20 ± 0.58		3.3
Sodium dichromate dihydrate	62.5	5	3.20 ± 0.41	0.0865	3.6
	125	5	3.00 ± 0.16	0.1333	3.2
	250	5	3.80 ± 0.37	0.0193	2.8
			P=0.031[f]		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.008 (ILS, 1990)
[d] Percentage of polychromatic erythrocytes (reticulocytes) among total erythrocytes
[e] Untreated control
[f] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE B4
Frequency of Micronuclei in Normochromatic Erythrocytes in Peripheral Blood of Male BALB/c Mice Following Administration of Sodium Dichromate Dihydrate in Drinking Water for 3 Months (Study 2)[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[d] (%)
Water[e]	0	5	4.70 ± 0.46		3.7
Sodium dichromate dihydrate	62.5	5	3.90 ± 0.48	0.8063	4.0
	125	5	3.30 ± 0.80	0.9416	3.3
	250	5	4.20 ± 0.34	0.7024	3.5
			P=0.680[f]		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.008 (ILS, 1990)
[d] Percentage of polychromatic erythrocytes (reticulocytes) among total erythrocytes
[e] Untreated control
[f] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE B5
Frequency of Micronuclei in Normochromatic Erythrocytes in Peripheral Blood of Male *am3*-C57BL/6 Mice Following Administration of Sodium Dichromate Dihydrate in Drinking Water for 3 Months (Study 2)[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[d] (%)
Water[e]	0	10	1.65 ± 0.24		2.9
Sodium dichromate dihydrate	62.5	10	2.50 ± 0.17	0.0391	2.8
	125	10	3.05 ± 0.26	0.0025	2.9
	250	9	3.72 ± 0.53	0.0001	2.6
			P<0.001[f]		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.
[b] Mean ± standard error
[c] Pairwise comparison with the untreated control group; significant at P≤0.008 (ILS, 1990)
[d] Percentage of polychromatic erythrocytes (reticulocytes) among total erythrocytes
[e] Untreated control
[f] Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX C
CLINICAL PATHOLOGY RESULTS

TABLE C1 Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1) C-2

TABLE C2 Hematology Data for B6C3F_1 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1) C-11

TABLE C3 Hematology and Clinical Chemistry Data for Male B6C3F_1 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2) C-12

TABLE C4 Hematology and Clinical Chemistry Data for Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2) C-13

TABLE C5 Hematology and Clinical Chemistry Data for Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2) C-14

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
Hematology						
n	10	10	10	10	10	10
Hematocrit (automated) (%)						
Day 5	45.8 ± 1.0	45.0 ± 0.8	45.2 ± 0.9	43.8 ± 0.8	44.6 ± 0.6	46.2 ± 0.7
Day 23	48.5 ± 0.7	45.0 ± 1.0*	34.3 ± 1.8**	28.0 ± 1.4**	24.3 ± 0.9**	21.1 ± 1.6**
Week 14	46.0 ± 0.3	45.5 ± 0.4	45.3 ± 0.3	44.9 ± 0.7	43.1 ± 0.5**	30.8 ± 1.9**
Hematocrit (manual) (%)						
Day 5	45.6 ± 1.1	45.1 ± 0.7	45.0 ± 0.8	44.0 ± 0.8	44.5 ± 0.6	45.9 ± 0.7
Day 23	48.0 ± 0.5	44.7 ± 0.7**	39.8 ± 0.8**	36.2 ± 1.0**	34.4 ± 0.5**	32.3 ± 1.1**
Week 14	45.7 ± 0.2	45.2 ± 0.4	45.2 ± 0.3	44.8 ± 0.7	42.9 ± 0.4**	36.9 ± 0.8**
Hemoglobin (g/dL)						
Day 5	15.3 ± 0.4	15.0 ± 0.2	15.1 ± 0.3	14.9 ± 0.3	15.1 ± 0.2	15.7 ± 0.2
Day 23	15.9 ± 0.1	14.2 ± 0.2**	12.0 ± 0.3**	10.9 ± 0.3**	10.3 ± 0.3**	9.2 ± 0.3**
Week 14	15.3 ± 0.1	15.2 ± 0.1	15.0 ± 0.1	14.4 ± 0.2**	13.3 ± 0.2**	10.9 ± 0.3**
Erythrocytes (10^6/µL)						
Day 5	7.27 ± 0.17	7.30 ± 0.12	7.46 ± 0.14	7.36 ± 0.13	7.43 ± 0.10	7.70 ± 0.10
Day 23	7.94 ± 0.10	8.38 ± 0.11	7.13 ± 0.35*	6.03 ± 0.28**	5.25 ± 0.19**	4.54 ± 0.33**
Week 14	8.88 ± 0.05	9.04 ± 0.09*	9.25 ± 0.07**	10.15 ± 0.22**	10.87 ± 0.07**	8.52 ± 0.45**
Reticulocytes (10^6/µL)						
Day 5	0.63 ± 0.02	0.52 ± 0.01**	0.31 ± 0.02**	0.19 ± 0.02**	0.17 ± 0.03**	0.10 ± 0.01**
Day 23	0.26 ± 0.01	0.40 ± 0.01**	0.39 ± 0.03**	0.32 ± 0.02	0.27 ± 0.01	0.27 ± 0.02
Week 14	0.23 ± 0.00	0.24 ± 0.00	0.22 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	0.31 ± 0.01**
Reticulocytes (%)						
Day 5	8.65 ± 0.39	7.07 ± 0.17**	4.15 ± 0.25**	2.60 ± 0.33**	2.30 ± 0.33**	1.27 ± 0.14**
Day 23	3.26 ± 0.15	4.83 ± 0.18**	5.38 ± 0.34**	5.29 ± 0.27**	5.16 ± 0.34**	6.00 ± 0.35**
Week 14	2.56 ± 0.04	2.61 ± 0.05	2.36 ± 0.06	2.34 ± 0.05	2.13 ± 0.08**	3.79 ± 0.32
Nucleated erythrocytes (10^3/µL)						
Day 5	0.20 ± 0.13	0.30 ± 0.15	0.20 ± 0.13	0.30 ± 0.15	0.10 ± 0.10	0.40 ± 0.16
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.70 ± 0.26**	1.30 ± 0.40**	1.90 ± 0.64**	2.70 ± 0.78**
Week 14	0.10 ± 0.10	0.30 ± 0.15	0.10 ± 0.10	0.10 ± 0.10	0.20 ± 0.13	1.20 ± 0.29**
Mean cell volume (fL)						
Day 5	63.1 ± 0.2	61.7 ± 0.2**	60.6 ± 0.2**	59.5 ± 0.2**	60.0 ± 0.2**	60.0 ± 0.3**
Day 23	61.1 ± 0.5	53.6 ± 0.6**	48.0 ± 0.4**	46.4 ± 0.6**	46.2 ± 0.3**	46.4 ± 0.5**
Week 14	51.8 ± 0.1	50.3 ± 0.2**	49.0 ± 0.1**	44.4 ± 1.0**	39.7 ± 0.5**	36.0 ± 0.4**
Mean cell hemoglobin (pg)						
Day 5	21.0 ± 0.1	20.5 ± 0.1**	20.3 ± 0.1**	20.3 ± 0.1**	20.4 ± 0.1**	20.4 ± 0.1**
Day 23	20.1 ± 0.2	16.9 ± 0.2**	17.2 ± 0.7**	18.2 ± 0.4	19.7 ± 0.3	20.7 ± 0.6
Week 14	17.3 ± 0.1	16.9 ± 0.1**	16.2 ± 0.1**	14.2 ± 0.4**	12.3 ± 0.2**	13.0 ± 0.5**
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.3 ± 0.2	33.3 ± 0.2	33.5 ± 0.1	34.1 ± 0.1**	34.0 ± 0.1**	34.0 ± 0.1**
Day 23	32.9 ± 0.4	31.6 ± 0.3	35.9 ± 1.7	39.2 ± 0.9**	42.8 ± 0.7**	44.6 ± 1.3**
Week 14	33.4 ± 0.1	34.0 ± 0.1	33.1 ± 0.2	32.0 ± 0.2**	31.0 ± 0.2**	36.3 ± 1.8*
Platelets (10^3/µL)						
Day 5	913.6 ± 28.9	943.0 ± 21.7	1,084.0 ± 51.6**	1,222.2 ± 46.4**	1,239.0 ± 43.2**	1,286.8 ± 36.2**
Day 23	745.2 ± 22.2	1,065.3 ± 67.9**	2,768.6 ± 328.5**	3,504.7 ± 235.0**	4,226.0 ± 204.5**	4,688.8 ± 242.7**
Week 14	618.6 ± 20.0	736.1 ± 11.5	604.3 ± 24.5	909.8 ± 119.1**	1,743.1 ± 178.0**	5,123.0 ± 638.9**
Platelet estimates (10^3/µL)						
Day 23	1,302.0 ± 68.9	1,537.2 ± 102.7	1,957.2 ± 106.2**	1,900.5 ± 170.3**	1,917.3 ± 83.7**	2,083.2 ± 158.5**
Week 14	676.2 ± 32.8	674.1 ± 25.3	636.3 ± 24.1	663.6 ± 21.5	678.3 ± 29.2	783.3 ± 28.5

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (continued)						
Hematology (continued)						
n	10	10	10	10	10	10
Leukocytes (10^3/μL)						
Day 5	9.04 ± 0.29	9.26 ± 0.27	9.33 ± 0.39	9.84 ± 0.26	9.24 ± 0.36	11.08 ± 0.17**
Day 23	10.37 ± 0.69	10.94 ± 0.58	11.11 ± 0.38	10.37 ± 0.74	11.91 ± 0.51	11.92 ± 0.56
Week 14	8.54 ± 0.28	9.38 ± 0.55	9.01 ± 0.46	9.49 ± 0.46	10.79 ± 0.52**	11.27 ± 0.80**
Segmented neutrophils (10^3/μL)						
Day 5	0.84 ± 0.02	0.91 ± 0.03	0.93 ± 0.04	0.89 ± 0.03	0.88 ± 0.05	1.51 ± 0.14**
Day 23	0.86 ± 0.06	0.92 ± 0.06	0.92 ± 0.05	0.76 ± 0.08	0.83 ± 0.04	1.38 ± 0.15
Week 14	1.22 ± 0.04	1.41 ± 0.05	1.30 ± 0.06	1.47 ± 0.09	1.27 ± 0.03	2.21 ± 0.19**
Lymphocytes (10^3/μL)						
Day 5	7.81 ± 0.28	7.99 ± 0.26	8.04 ± 0.37	8.55 ± 0.22	7.96 ± 0.30	9.09 ± 0.19**
Day 23	9.15 ± 0.58	9.66 ± 0.50	9.85 ± 0.34	9.31 ± 0.64	10.73 ± 0.49	10.12 ± 0.47
Week 14	7.02 ± 0.26	7.62 ± 0.51	7.39 ± 0.44	7.67 ± 0.41	9.09 ± 0.51**	8.70 ± 0.67*
Monocytes (10^3/μL)						
Day 5	0.21 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.19 ± 0.02	0.20 ± 0.02	0.26 ± 0.02
Day 23	0.17 ± 0.03	0.16 ± 0.02	0.17 ± 0.01	0.14 ± 0.02	0.17 ± 0.01	0.25 ± 0.03*
Week 14	0.13 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.19 ± 0.02*	0.19 ± 0.02**	0.19 ± 0.02**
Basophils (10^3/μL)						
Day 5	0.054 ± 0.004	0.051 ± 0.003	0.055 ± 0.005	0.070 ± 0.006	0.055 ± 0.003	0.074 ± 0.003**
Day 23	0.054 ± 0.006	0.046 ± 0.005	0.040 ± 0.004	0.035 ± 0.005	0.047 ± 0.004	0.037 ± 0.003
Week 14	0.045 ± 0.009	0.045 ± 0.004	0.041 ± 0.005	0.035 ± 0.005	0.043 ± 0.005	0.036 ± 0.003
Eosinophils (10^3/μL)						
Day 5	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
Day 23	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.00*	0.02 ± 0.00**	0.02 ± 0.00**	0.03 ± 0.00**
Week 14	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01
Large unstained cells (10^3/mm^3)						
Day 5	0.107 ± 0.012	0.092 ± 0.006	0.104 ± 0.009	0.120 ± 0.009	0.119 ± 0.019	0.134 ± 0.017
Day 23	0.097 ± 0.017	0.112 ± 0.011	0.112 ± 0.011	0.101 ± 0.014	0.110 ± 0.012	0.108 ± 0.008
Week 14	0.058 ± 0.009	0.062 ± 0.008	0.058 ± 0.006	0.061 ± 0.006	0.100 ± 0.015**	0.077 ± 0.007*
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 5	14.6 ± 0.5	13.7 ± 0.4	14.2 ± 0.3	14.9 ± 0.3	14.8 ± 0.2	13.7 ± 0.4
Day 23	14.0 ± 0.6	14.2 ± 0.4	14.8 ± 0.4	15.5 ± 0.4*	17.2 ± 0.4**	16.5 ± 0.9**
Week 14	14.7 ± 0.3	16.9 ± 0.8	17.3 ± 1.0	16.5 ± 0.8	20.2 ± 0.5**	14.9 ± 0.4
Creatinine (mg/dL)						
Day 5	0.53 ± 0.02	0.52 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.50 ± 0.00	0.50 ± 0.00
Day 23	0.58 ± 0.01	0.53 ± 0.02	0.57 ± 0.02	0.53 ± 0.02	0.55 ± 0.02	0.51 ± 0.01**
Week 14	0.66 ± 0.02	0.60 ± 0.02	0.63 ± 0.02	0.67 ± 0.02	0.61 ± 0.02	0.59 ± 0.01*
Glucose (mg/dL)						
Day 5	146 ± 3	145 ± 3	147 ± 2	147 ± 3	143 ± 2	140 ± 2
Day 23	148 ± 6	145 ± 3	147 ± 3	153 ± 4	148 ± 4	149 ± 2
Week 14	155 ± 10	154 ± 7	155 ± 9	154 ± 8	141 ± 5	145 ± 5
Sodium (mEq/L)						
Day 5	146 ± 0	146 ± 1	147 ± 1	147 ± 0	148 ± 0**	150 ± 1**
Day 23	145 ± 1	145 ± 1	145 ± 1	145 ± 1	145 ± 1	145 ± 1
Week 14	155 ± 1	154 ± 1	155 ± 1	155 ± 1	155 ± 1	155 ± 1

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Potassium (mEq/L)						
Day 5	7.0 ± 0.1	6.9 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
Day 23	5.5 ± 0.2	5.4 ± 0.1	5.9 ± 0.2	5.8 ± 0.1	5.8 ± 0.2	5.8 ± 0.1
Week 14	5.1 ± 0.2	5.2 ± 0.2	5.4 ± 0.1	5.2 ± 0.2	5.5 ± 0.2	5.8 ± 0.1**
Chloride (mEq/L)						
Day 5	101 ± 0	101 ± 1	102 ± 0*	103 ± 0**	104 ± 0**	105 ± 0**
Day 23	99 ± 1	100 ± 1	100 ± 0	101 ± 1	100 ± 0	101 ± 1
Week 14	106 ± 1	107 ± 1	106 ± 1	108 ± 1	107 ± 1	109 ± 1
Calcium (mg/dL)						
Day 5	12.47 ± 0.10	12.42 ± 0.14	12.36 ± 0.08	11.98 ± 0.07**	11.99 ± 0.11**	11.90 ± 0.11**
Day 23	11.85 ± 0.07	11.82 ± 0.14	11.79 ± 0.12	11.81 ± 0.09	11.70 ± 0.09	11.90 ± 0.11
Week 14	11.67 ± 0.09	11.34 ± 0.09	11.65 ± 0.09	11.49 ± 0.09	11.35 ± 0.10	11.63 ± 0.08
Phosphorus (mg/dL)						
Day 5	11.5 ± 0.1	11.8 ± 0.1	11.8 ± 0.1	11.4 ± 0.1	11.6 ± 0.1	11.2 ± 0.1
Day 23	9.8 ± 0.4	9.5 ± 0.2	9.9 ± 0.2	10.1 ± 0.2	10.6 ± 0.3*	10.6 ± 0.1**
Week 14	6.5 ± 0.2	6.6 ± 0.3	6.3 ± 0.2	6.6 ± 0.3	6.4 ± 0.1	6.8 ± 0.3
Total protein (g/dL)						
Day 5	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.7 ± 0.1*
Day 23	6.1 ± 0.1	5.9 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	5.9 ± 0.1*	5.8 ± 0.1**
Week 14	6.7 ± 0.1	6.5 ± 0.1	6.7 ± 0.0	6.6 ± 0.1	6.5 ± 0.1	6.4 ± 0.1
Albumin (g/dL)						
Day 5	3.9 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.8 ± 0.0	3.8 ± 0.0	3.7 ± 0.0**
Day 23	4.0 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.8 ± 0.0
Week 14	4.3 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.1	4.4 ± 0.0	4.3 ± 0.0
Cholesterol (mg/dL)						
Day 5	112 ± 3	106 ± 2	103 ± 2**	103 ± 1**	103 ± 2**	103 ± 3**
Day 23	86 ± 3	69 ± 2**	78 ± 2	76 ± 2	71 ± 1**	77 ± 2
Week 14	89 ± 2	95 ± 2	86 ± 4	65 ± 2**	86 ± 3*	71 ± 2**
Triglycerides (mg/dL)						
Day 5	119 ± 9	111 ± 11	110 ± 7	119 ± 6	109 ± 8	112 ± 10
Day 23	212 ± 12	168 ± 7	191 ± 9	157 ± 16**	146 ± 7**	109 ± 7**
Week 14	170 ± 9	169 ± 8	172 ± 15	170 ± 13	164 ± 12	98 ± 8**
Alanine aminotransferase (IU/L)						
Day 5	48 ± 1	55 ± 2**	69 ± 2**	73 ± 3**	73 ± 3**	70 ± 2**
Day 23	44 ± 1	63 ± 5**	65 ± 2**	69 ± 2**	75 ± 3**	67 ± 3**
Week 14	98 ± 6[b]	274 ± 30**	461 ± 102**	447 ± 121**	740 ± 81**	191 ± 17**
Alkaline phosphatase (IU/L)						
Day 5	641 ± 11	620 ± 16	589 ± 9**	573 ± 10**	554 ± 15**	529 ± 12**
Day 23	442 ± 11	443 ± 13	421 ± 16	399 ± 5**	378 ± 8**	348 ± 19**
Week 14	181 ± 4	157 ± 6**	157 ± 3**	147 ± 3**	133 ± 4**	136 ± 4**
Creatine kinase (IU/L)						
Day 5	407 ± 23	434 ± 49	495 ± 30	486 ± 36	533 ± 43**	520 ± 21**
Day 23	586 ± 35	582 ± 62	663 ± 67	636 ± 74[b]	810 ± 73	656 ± 82
Week 14	214 ± 26	286 ± 32	291 ± 36	364 ± 23**	413 ± 16**	374 ± 44**
Sorbitol dehydrogenase (IU/L)						
Day 5	19 ± 1	20 ± 1	17 ± 1	18 ± 1	17 ± 1	17 ± 1
Day 23	20 ± 2	16 ± 1	16 ± 1	16 ± 2	16 ± 1	16 ± 1
Week 14	31 ± 2[b]	55 ± 5**	110 ± 24**	102 ± 24**	173 ± 20**	59 ± 6**

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
5′-Nucleotidase (IU/L)						
Day 5	40 ± 1	39 ± 1	38 ± 1	38 ± 1	37 ± 1*	33 ± 1**
Day 23	37 ± 2	34 ± 1	39 ± 2	38 ± 1	36 ± 1	39 ± 2
Week 14	42 ± 0	37 ± 1**	42 ± 1	40 ± 1	38 ± 1	41 ± 1
Bile acids (µmol/L)						
Day 5	29.0 ± 1.6	25.9 ± 1.8	26.6 ± 1.6	32.1 ± 1.9	28.0 ± 1.7	26.5 ± 1.8
Day 23	23.4 ± 1.6	23.2 ± 2.4	29.1 ± 1.7	30.4 ± 3.1	29.8 ± 2.2	32.8 ± 1.6**
Week 14	22.0 ± 2.2	24.0 ± 3.4	34.5 ± 7.0	32.6 ± 5.3	45.3 ± 2.8**	28.1 ± 2.0**
Urinalysis (Day 17)						
n	10	10	10	10	10	9
Creatinine (mg/dL)	85.20 ± 5.33	81.28 ± 7.84	100.32 ± 4.71	104.08 ± 4.72*	124.76 ± 5.13**	115.60 ± 10.24**
Glucose (mg/dL)	38 ± 3	32 ± 2	45 ± 4	43 ± 3	59 ± 3**	60 ± 6**
Normalized glucose	0.45 ± 0.03	0.41 ± 0.03	0.45 ± 0.03	0.41 ± 0.03	478 ± 0.02	0.51 ± 0.02
Protein (mg/dL)	83 ± 15	88 ± 14	130 ± 16*	138 ± 13*	136 ± 11*	118 ± 14*
Normalized protein	0.96 ± 0.15	1.04 ± 0.12	1.30 ± 0.15	1.37 ± 0.16	1.10 ± 0..96	1.02 ± 0.13
Alkaline phosphatase (IU/L)	165 ± 18	136 ± 20	160 ± 12	155 ± 13	197 ± 16	172 ± 24
Normalized alkaline phosphatase	1.95 ± 0.17	1.65 ± 0.12	1.59 ± 0.07	1.51 ± 0.14	1.582 ± 0.10	1.46 ± 0.11
Aspartate aminotransferase (IU/L)	6 ± 1[b]	5 ± 0	7 ± 1	8 ± 1*	12 ± 2**	12 ± 1**
Normalized aspartate aminotransferase	0.07 ± 0.01[b]	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.00	0.09 ± 0.01	0.10 ± 0.01**
N-acetyl-β-D-glucosaminidase (IU/L)	9 ± 1	10 ± 1	11 ± 0**	12 ± 1**	13 ± 1**	14 ± 1**
Normalized N-acetyl-β-D-glucosaminidase	0.11 ± 0.00	0.12 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.11 ± 0.00	0.12 ± 0.01
Volume (mL)	3.9 ± 0.4	3.5 ± 0.4	2.9 ± 0.5	3.0 ± 0.3	2.2 ± 0.2**	1.5 ± 0.2**
Specific gravity	1.047 ± 0.003	1.046 ± 0.003	1.059 ± 0.002**	1.058 ± 0.002**	1.063 ± 0.003**	1.065 ± 0.005**
pH	6.85 ± 0.11	6.80 ± 0.11	6.80 ± 0.08	7.05 ± 0.24	7.10 ± 0.13	7.33 ± 0.33

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	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female						
Hematology						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	9	10	10	10	9
Hematocrit (automated) (%)						
Day 5	48.2 ± 1.3	48.4 ± 0.8	47.4 ± 1.3	46.8 ± 1.2	48.7 ± 0.6	48.5 ± 1.0
Day 23	47.7 ± 0.4	45.9 ± 0.9	35.2 ± 1.1**	29.6 ± 2.0**	24.1 ± 1.2**	19.5 ± 0.7**
Week 14	44.2 ± 0.3	45.8 ± 0.2	44.0 ± 0.2	42.8 ± 0.3*	42.8 ± 0.4*	38.4 ± 0.6**
Hematocrit (manual) (%)						
Day 5	47.8 ± 1.1	48.5 ± 0.8	47.2 ± 1.3	46.3 ± 1.3	48.3 ± 0.6	47.7 ± 1.0
Day 23	48.0 ± 0.4	46.6 ± 0.9	42.9 ± 0.8**	39.2 ± 0.7**	37.2 ± 0.7**	33.4 ± 0.6**
Week 14	44.6 ± 0.4	45.2 ± 0.1	44.1 ± 0.3	42.9 ± 0.2**	42.6 ± 0.5**	38.3 ± 0.5**
Hemoglobin (g/dL)						
Day 5	16.1 ± 0.4	16.2 ± 0.3	15.9 ± 0.4	15.7 ± 0.4	16.3 ± 0.2	16.4 ± 0.3
Day 23	15.9 ± 0.1	14.7 ± 0.3**	13.0 ± 0.3**	11.8 ± 0.3**	10.9 ± 0.2**	9.7 ± 0.2**
Week 14	15.2 ± 0.1	15.4 ± 0.1	14.9 ± 0.1	14.3 ± 0.1**	14.1 ± 0.2**	12.0 ± 0.2**
Erythrocytes (10^6/µL)						
Day 5	7.66 ± 0.21	7.77 ± 0.13	7.74 ± 0.20	7.68 ± 0.17	8.00 ± 0.08	8.03 ± 0.15
Day 23	7.82 ± 0.09	8.52 ± 0.14	7.22 ± 0.19	6.32 ± 0.36**	5.27 ± 0.23**	4.21 ± 0.16**
Week 14	8.30 ± 0.06	8.60 ± 0.05**	8.40 ± 0.04*	8.47 ± 0.04*	8.93 ± 0.11**	9.62 ± 0.10**
Reticulocytes (10^6/µL)						
Day 5	0.50 ± 0.02	0.43 ± 0.02*	0.33 ± 0.02**	0.22 ± 0.03**	0.24 ± 0.03**	0.12 ± 0.01**
Day 23	0.21 ± 0.01	0.27 ± 0.02	0.32 ± 0.03*	0.30 ± 0.03*	0.23 ± 0.02	0.22 ± 0.03
Week 14	0.17 ± 0.00	0.22 ± 0.01**	0.21 ± 0.00**	0.21 ± 0.01**	0.21 ± 0.01**	0.24 ± 0.02**
Reticulocytes (%)						
Day 5	6.61 ± 0.35	5.51 ± 0.23*	4.30 ± 0.24**	2.92 ± 0.34**	2.97 ± 0.33**	1.55 ± 0.20**
Day 23	2.72 ± 0.15	3.18 ± 0.23	4.40 ± 0.41**	4.78 ± 0.28**	4.43 ± 0.31**	5.10 ± 0.49**
Week 14	2.04 ± 0.05	2.60 ± 0.08**	2.47 ± 0.04*	2.51 ± 0.09**	2.37 ± 0.10	2.52 ± 0.17*
Nucleated erythrocytes (10^3/µL)						
Day 5	0.20 ± 0.13	0.30 ± 0.15	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.33 ± 0.24	0.38 ± 0.18	0.33 ± 0.17	0.40 ± 0.16	1.11 ± 0.39**
Week 14	0.30 ± 0.21	0.11 ± 0.11	0.40 ± 0.22	0.20 ± 0.20	0.00 ± 0.00	0.11 ± 0.11
Mean cell volume (fL)						
Day 5	63.0 ± 0.3	62.3 ± 0.3	61.2 ± 0.3**	60.8 ± 0.4**	60.9 ± 0.3**	60.4 ± 0.3**
Day 23	61.1 ± 0.4	53.9 ± 0.5**	48.8 ± 0.5**	46.6 ± 0.6**	45.7 ± 0.4**	46.5 ± 0.5**
Week 14	53.3 ± 0.1	53.3 ± 0.1	52.4 ± 0.2**	50.5 ± 0.3**	48.0 ± 0.9**	40.0 ± 0.7**
Mean cell hemoglobin (pg)						
Day 5	21.0 ± 0.1	20.8 ± 0.1	20.5 ± 0.1*	20.4 ± 0.1**	20.4 ± 0.1**	20.5 ± 0.1**
Day 23	20.4 ± 0.1	17.3 ± 0.2	18.0 ± 0.3	18.9 ± 0.7	21.0 ± 0.6	23.1 ± 0.5
Week 14	18.4 ± 0.1	17.9 ± 0.1**	17.8 ± 0.1**	16.9 ± 0.1**	15.9 ± 0.4**	12.5 ± 0.3**
Mean cell hemoglobin concentration (g/dL)						
Day 5	33.4 ± 0.2	33.4 ± 0.2	33.5 ± 0.2	33.6 ± 0.2	33.5 ± 0.2	33.9 ± 0.2
Day 23	33.3 ± 0.1	32.1 ± 0.1	37.0 ± 0.7*	40.8 ± 1.8**	45.8 ± 1.4**	49.6 ± 0.9**
Week 14	34.5 ± 0.1	33.7 ± 0.1**	33.9 ± 0.1**	33.5 ± 0.1**	33.0 ± 0.3**	31.2 ± 0.2**
Platelets (10^3/µL)						
Day 5	856.8 ± 36.4	872.1 ± 20.5	958.3 ± 34.2	1,045.8 ± 47.5*	1,003.3 ± 45.1*	1,002.1 ± 41.5*
Day 23	611.5 ± 43.7	1,156.3 ± 76.4**	2,808.8 ± 198.5**	3,295.0 ± 349.7**	4,318.4 ± 234.9**	5,132.8 ± 247.0**
Week 14	588.9 ± 17.1	605.8 ± 17.1	574.8 ± 21.3	528.2 ± 14.1	619.3 ± 55.4	1,524.9 ± 193.3**

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Hematology (continued)						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	9	10	10	10	9
Platelet estimates ($10^3/\mu L$)						
Day 23	1,404.9 ± 50.8	1,213.3 ± 43.4	1,433.3 ± 62.0	1,369.7 ± 61.6	1,365.0 ± 46.1	1,449.0 ± 69.8
Week 14	1,022.7 ± 58.1	994.0 ± 55.0	997.5 ± 52.5	942.9 ± 42.5	858.9 ± 43.5	1,033.7 ± 79.9
Leukocytes ($10^3/\mu L$)						
Day 5	9.53 ± 0.46	9.36 ± 0.35	9.34 ± 0.37	9.50 ± 0.53	9.88 ± 0.48	10.00 ± 0.37
Day 23	9.66 ± 0.50	10.77 ± 0.42	12.07 ± 0.63*	11.17 ± 0.95*	11.76 ± 0.29*	12.01 ± 0.33**
Week 14	7.09 ± 0.45	7.16 ± 0.29	7.59 ± 0.25	7.25 ± 0.55	8.03 ± 0.40	10.91 ± 0.58**
Segmented neutrophils ($10^3/\mu L$)						
Day 5	0.94 ± 0.05	0.94 ± 0.05	0.97 ± 0.06	0.98 ± 0.06	1.00 ± 0.06	1.35 ± 0.08**
Day 23	0.98 ± 0.05	1.10 ± 0.07	0.98 ± 0.05	0.83 ± 0.10	0.81 ± 0.03	1.40 ± 0.08*
Week 14	1.26 ± 0.09	1.04 ± 0.04	1.23 ± 0.05	1.31 ± 0.11	1.47 ± 0.11	2.83 ± 0.22**
Lymphocytes ($10^3/\mu L$)						
Day 5	8.18 ± 0.38	8.04 ± 0.30	8.00 ± 0.33	8.13 ± 0.47	8.46 ± 0.41	8.15 ± 0.32
Day 23	8.32 ± 0.46	9.28 ± 0.36	10.65 ± 0.56**	9.96 ± 0.84*	10.57 ± 0.27**	10.16 ± 0.31**
Week 14	5.55 ± 0.37	5.81 ± 0.28	6.06 ± 0.23	5.63 ± 0.46	6.19 ± 0.32	7.60 ± 0.46*
Monocytes ($10^3/\mu L$)						
Day 5	0.22 ± 0.03	0.21 ± 0.02	0.20 ± 0.02	0.22 ± 0.02	0.23 ± 0.02	0.26 ± 0.01
Day 23	0.16 ± 0.01	0.18 ± 0.02	0.22 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.24 ± 0.02**
Week 14	0.12 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01*	0.18 ± 0.02**	0.26 ± 0.02**
Basophils ($10^3/\mu L$)						
Day 5	0.063 ± 0.007	0.054 ± 0.006	0.054 ± 0.003	0.057 ± 0.005	0.063 ± 0.006	0.071 ± 0.009
Day 23	0.061 ± 0.005	0.054 ± 0.006	0.059 ± 0.005	0.044 ± 0.006	0.049 ± 0.004	0.050 ± 0.003
Week 14	0.034 ± 0.002	0.042 ± 0.003	0.047 ± 0.007	0.049 ± 0.010	0.049 ± 0.006	0.056 ± 0.008
Eosinophils ($10^3/\mu L$)						
Day 5	0.04 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Day 23	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.03 ± 0.00**	0.04 ± 0.01**
Week 14	0.06 ± 0.00	0.07 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01
Large unstained cells ($10^3/mm^3$)						
Day 5	0.100 ± 0.017	0.099 ± 0.008	0.084 ± 0.008	0.096 ± 0.010	0.101 ± 0.009	0.140 ± 0.011**
Day 23	0.081 ± 0.008	0.100 ± 0.009	0.110 ± 0.021	0.112 ± 0.014	0.119 ± 0.016*	0.123 ± 0.007**
Week 14	0.067 ± 0.006	0.066 ± 0.005	0.069 ± 0.005	0.070 ± 0.008	0.088 ± 0.008	0.093 ± 0.013
Clinical Chemistry						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 5	13.2 ± 0.3	13.8 ± 0.4	13.9 ± 0.4	14.1 ± 0.4	14.9 ± 0.5**	15.1 ± 0.2**
Day 23	17.1 ± 0.4	17.6 ± 0.6	19.0 ± 1.3	18.6 ± 0.9	18.6 ± 0.5	18.2 ± 0.5
Week 14	16.4 ± 0.3	19.8 ± 0.6**	17.2 ± 0.4*	18.6 ± 0.6**	19.0 ± 0.7**	20.7 ± 0.8**

TABLE C1
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	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	10	10	10	10	10
Creatinine (mg/dL)						
Day 5	0.51 ± 0.01	0.50 ± 0.00	0.50 ± 0.02	0.47 ± 0.02	0.48 ± 0.01	0.48 ± 0.02
Day 23	0.57 ± 0.02	0.50 ± 0.02**	0.50 ± 0.00**	0.50 ± 0.01**	0.49 ± 0.01**	0.47 ± 0.02**
Week 14	0.55 ± 0.02	0.51 ± 0.02	0.53 ± 0.02	0.53 ± 0.02	0.54 ± 0.02	0.52 ± 0.01
Glucose (mg/dL)						
Day 5	146 ± 5	141 ± 4	145 ± 4	141 ± 6	135 ± 3	130 ± 5*
Day 23	149 ± 4	144 ± 6	146 ± 8	146 ± 6	149 ± 3	140 ± 3
Week 14	139 ± 6	134 ± 3	144 ± 7	152 ± 6	146 ± 7	144 ± 8
Sodium (mEq/L)						
Day 5	144 ± 1	144 ± 1	144 ± 1	145 ± 0	145 ± 0	146 ± 0
Day 23	147 ± 1	146 ± 1	147 ± 0	146 ± 1	146 ± 0	145 ± 1**
Week 14	147 ± 1	147 ± 0	145 ± 1	147 ± 1	148 ± 1	146 ± 1
Potassium (mEq/L)						
Day 5	6.4 ± 0.1	6.2 ± 0.2	6.4 ± 0.2	6.3 ± 0.2	6.5 ± 0.2	6.2 ± 0.2
Day 23	5.3 ± 0.1	5.5 ± 0.2	6.0 ± 0.1**	5.7 ± 0.2	5.6 ± 0.2	5.8 ± 0.1*
Week 14	4.7 ± 0.2	5.2 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.1 ± 0.1
Chloride (mEq/L)						
Day 5	100 ± 1	100 ± 0	101 ± 1	102 ± 1	101 ± 0	102 ± 1**
Day 23	102 ± 1	102 ± 1	103 ± 1	103 ± 1	102 ± 0	103 ± 1
Week 14	102 ± 1	101 ± 0	100 ± 1	103 ± 1	103 ± 1	103 ± 1
Calcium (mg/dL)						
Day 5	12.89 ± 0.13	12.53 ± 0.12*	12.45 ± 0.13*	12.35 ± 0.20*	12.33 ± 0.15*	12.10 ± 0.09**
Day 23	12.03 ± 0.09	11.82 ± 0.14	11.81 ± 0.12	11.69 ± 0.10	11.76 ± 0.08	11.62 ± 0.10
Week 14	11.65 ± 0.10	11.46 ± 0.09	11.78 ± 0.08	11.46 ± 0.09	11.60 ± 0.10	11.30 ± 0.06
Phosphorus (mg/dL)						
Day 5	11.2 ± 0.2	10.9 ± 0.2	11.0 ± 0.2	11.1 ± 0.3	11.5 ± 0.2	10.4 ± 0.3
Day 23	8.9 ± 0.2	9.6 ± 0.3	9.5 ± 0.3	9.3 ± 0.3	10.0 ± 0.3**	10.0 ± 0.2**
Week 14	4.8 ± 0.3	5.7 ± 0.4	5.0 ± 0.3	4.0 ± 0.3	4.8 ± 0.4	5.5 ± 0.3
Total protein (g/dL)						
Day 5	5.9 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.7 ± 0.0
Day 23	6.2 ± 0.1	6.1 ± 0.1	6.0 ± 0.2	5.9 ± 0.1*	5.9 ± 0.1*	5.7 ± 0.1**
Week 14	6.9 ± 0.1	6.5 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.3 ± 0.1**
Albumin (g/dL)						
Day 5	3.9 ± 0.1	3.9 ± 0.0	3.9 ± 0.1	3.9 ± 0.1	3.9 ± 0.1	3.8 ± 0.0
Day 23	4.2 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.0	3.9 ± 0.0**
Week 14	4.8 ± 0.1	4.5 ± 0.0*	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	4.4 ± 0.1**
Cholesterol (mg/dL)						
Day 5	106 ± 3	104 ± 4	98 ± 3	103 ± 3	104 ± 3	95 ± 4
Day 23	87 ± 2	86 ± 2	79 ± 6	82 ± 4*	78 ± 1**	78 ± 3**
Week 14	95 ± 2	111 ± 4	94 ± 2	87 ± 2	83 ± 2*	79 ± 2**
Triglycerides (mg/dL)						
Day 5	110 ± 12	115 ± 8	102 ± 9	86 ± 6	99 ± 8	116 ± 14
Day 23	94 ± 12	89 ± 7	95 ± 10	85 ± 11	63 ± 5	78 ± 8
Week 14	139 ± 18	116 ± 10	98 ± 9	81 ± 4**	76 ± 7**	59 ± 6**

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Clinical Chemistry (continued)						
n						
Day 5	10	10	10	10	10	10
Day 23	10	9	8	9	10	9
Week 14	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 5	43 ± 2	56 ± 2**	70 ± 3**	81 ± 1**	80 ± 2**	81 ± 3**
Day 23	33 ± 1	48 ± 4**	65 ± 4**	74 ± 4**	71 ± 2**	74 ± 3**
Week 14	64 ± 5	437 ± 68**	218 ± 27**	245 ± 30**	246 ± 37**	248 ± 22**
Alkaline phosphatase (IU/L)						
Day 5	535 ± 14	494 ± 13*	477 ± 13**	453 ± 11**	452 ± 14**	410 ± 15**
Day 23	357 ± 6	329 ± 6*	318 ± 11**	297 ± 4**	287 ± 6**	237 ± 10**
Week 14	166 ± 5	196 ± 5	124 ± 3**	113 ± 3**	111 ± 2**	107 ± 4**
Creatine kinase (IU/L)						
Day 5	370 ± 36	442 ± 50	458 ± 28	536 ± 54*	540 ± 49*	544 ± 52*
Day 23	397 ± 45	436 ± 56	638 ± 110	530 ± 72	561 ± 70	549 ± 105
Week 14	197 ± 23	311 ± 94	265 ± 23	296 ± 24**	359 ± 23**	432 ± 48**
Sorbitol dehydrogenase (IU/L)						
Day 5	23 ± 1	20 ± 1	20 ± 1	19 ± 1	19 ± 2	19 ± 1
Day 23	19 ± 1	18 ± 1	16 ± 1	14 ± 1**	13 ± 1**	15 ± 1**
Week 14	22 ± 2	101 ± 17**	65 ± 10**	81 ± 13**	96 ± 20**	103 ± 12**
5′-Nucleotidase (IU/L)						
Day 5	42 ± 1	39 ± 1	38 ± 1	37 ± 1	39 ± 2	35 ± 1**
Day 23	55 ± 2	53 ± 2	56 ± 2	53 ± 3	56 ± 2	53 ± 2
Week 14	43 ± 1	41 ± 1	42 ± 1	41 ± 1	44 ± 2	49 ± 1
Bile acids (μmol/L)						
Day 5	21.5 ± 1.7	20.2 ± 1.6	21.0 ± 1.4	24.3 ± 1.8	21.8 ± 1.8	20.0 ± 1.4
Day 23	22.9 ± 1.7	23.4 ± 1.9	19.9 ± 1.6	27.5 ± 1.5	27.5 ± 1.5	26.9 ± 4.0
Week 14	19.7 ± 2.5	50.4 ± 6.0**	39.9 ± 4.3**	35.3 ± 3.5	45.3 ± 5.6**	38.7 ± 3.2*
Urinalysis (Day 17)						
n	10	9	8	9	9	8
Creatinine (mg/dL)	89.28 ± 6.33	88.93 ± 5.33	118.30 ± 8.44*	110.49 ± 5.36*	112.27 ± 11.34*[d]	122.69 ± 8.81**[e]
Glucose (mg/dL)	28 ± 2	30 ± 2	46 ± 4**	42 ± 2**	50 ± 5**[e]	52 ± 5**[e]
Normalized glucose	0.32 ± 0.01	0.35 ± 0.01	0.38 ± 0.02*	0.39 ± 0.02**	0.43 ± 0.03**[d]	0.43 ± 0.03**[e]
Protein (mg/dL)	35 ± 3	37 ± 4	56 ± 6*	48 ± 4*	60 ± 9*[e]	59 ± 7**[e]
Normalized protein	0.39 ± 0.02	0.41 ± 0.02	0.47 ± 0.03	0.43 ± 0.02	0.47 ± 0.03[d]	0.47 ± 0.03[e]
Alkaline phosphatase (IU/L)	104 ± 10	95 ± 10	114 ± 13	113 ± 9	100 ± 15[c]	94 ± 11
Normalized alkaline phosphatase	1.16 ± 0.07	1.13 ± 0.14	0.96 ± 0.09	1.02 ± 0.05	0.92 ± 0.14[d]	0.79 ± 0.10*[e]
Aspartate aminotransferase (IU/L)	7 ± 1	6 ± 1	7 ± 1	10 ± 1	12 ± 1**	11 ± 1**
Normalized aspartate aminotransferase	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.10 ± 0.02	0.10 ± 0.01[d]	0.08 ± 0.01[e]

TABLE C1
Hematology, Clinical Chemistry, and Urinalysis Data for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Urinalysis (continued)						
n	10	9	8	9	9	8
N-acetyl-β-D-glucosaminidase (IU/L)	9 ± 1	9 ± 1[c]	13 ± 1**	12 ± 1**	12 ± 1**[c]	13 ± 1**
Normalized N-acetyl-β-D-glucosaminidase	0.10 ± 0.00	0.11 ± 0.00[c]	0.11 ± 0.00	0.11 ± 0.01	0.11 ± 0.01[d]	0.10 ± 0.01[e]
Volume (mL)	2.7 ± 0.3	2.6 ± 0.3	1.9 ± 0.3	10.4 ± 0.2**	1.0 ± 0.2**	0.8 ± 0.1**[b]
Specific gravity	1.054 ± 0.003	1.059 ± 0.004	1.082 ± 0.009**	1.079 ± 0.006**	1.092 ± 0.008**	1.087 ± 0.010**
pH	7.10 ± 0.16	7.06 ± 0.27	6.94 ± 0.15	7.17 ± 0.31	7.78 ± 0.31	7.75 ± 0.38

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] n=6
[e] n=7

TABLE C2
Hematology Data for B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
n	10	10	10	10	10	9
Hematocrit (automated) (%)	47.7 ± 1.2	45.4 ± 0.9	47.5 ± 1.0	46.3 ± 1.0	46.3 ± 1.0	45.5 ± 1.1
Hematocrit (manual) (%)	48.3 ± 1.1	46.2 ± 0.9	48.6 ± 0.9	46.8 ± 0.7	47.5 ± 0.9	46.8 ± 0.9
Hemoglobin (g/dL)	16.3 ± 0.4	15.5 ± 0.4	16.3 ± 0.4	15.8 ± 0.3	15.6 ± 0.3	15.4 ± 0.3
Erythrocytes (10^6/µL)	10.63 ± 0.28	10.31 ± 0.22	10.97 ± 0.23	10.84 ± 0.21	10.92 ± 0.25	10.95 ± 0.25
Reticulocytes (10^6/µL)	0.30 ± 0.01	0.27 ± 0.01*	0.29 ± 0.01	0.28 ± 0.00	0.28 ± 0.01	0.28 ± 0.01
Reticulocytes (%)	2.83 ± 0.09	2.61 ± 0.06	2.62 ± 0.06	2.54 ± 0.05*	2.53 ± 0.03**	2.56 ± 0.03*
Nucleated erythrocytes (10^3/µL)	0.10 ± 0.10	0.10 ± 0.10	0.20 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.33 ± 0.24
Mean cell volume (fL)	44.9 ± 0.3	44.1 ± 0.2*	43.3 ± 0.1**	42.7 ± 0.1**	42.4 ± 0.1**	41.6 ± 0.1**
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.0 ± 0.1**	14.8 ± 0.0**	14.6 ± 0.1**	14.4 ± 0.0**	14.1 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	34.1 ± 0.2	34.0 ± 0.2	34.3 ± 0.1	34.1 ± 0.1	33.8 ± 0.1	33.9 ± 0.1
Platelets (10^3/µL)	891.2 ± 45.6	1,032.5 ± 44.0	931.0 ± 57.8	909.0 ± 43.7	938.0 ± 37.6	961.0 ± 34.2
Leukocytes (10^3/µL)	4.29 ± 0.62	3.63 ± 0.48	3.99 ± 0.53	3.93 ± 0.47	4.07 ± 0.73	4.41 ± 0.50
Segmented neutrophils (10^3/µL)	0.47 ± 0.05	0.44 ± 0.07	0.58 ± 0.14	0.50 ± 0.08	0.45 ± 0.05	0.43 ± 0.05
Lymphocytes (10^3/µL)	3.62 ± 0.56	3.02 ± 0.41	3.23 ± 0.45	3.26 ± 0.43	3.41 ± 0.65	3.76 ± 0.45
Monocytes (10^3/µL)	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.06 ± 0.01
Basophils (10^3/µL)	0.017 ± 0.003	0.019 ± 0.005	0.014 ± 0.004	0.016 ± 0.002	0.017 ± 0.003	0.014 ± 0.003
Eosinophils (10^3/µL)	0.10 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.02	0.14 ± 0.03	0.12 ± 0.02
Large unstained cells (10^3/mm^3)	0.024 ± 0.005	0.012 ± 0.002	0.019 ± 0.005	0.016 ± 0.002	0.018 ± 0.006	0.019 ± 0.003
Female						
n	9	10	10	10	10	10
Hematocrit (automated) (%)	46.4 ± 0.4	46.1 ± 0.5	47.5 ± 0.6	47.1 ± 0.5	45.8 ± 0.6	45.5 ± 0.6
Hematocrit (manual) (%)	46.3 ± 0.4	46.0 ± 0.6	47.7 ± 0.5	47.0 ± 0.4	45.8 ± 0.4	45.7 ± 0.4
Hemoglobin (g/dL)	15.7 ± 0.1	15.5 ± 0.2	15.8 ± 0.1	15.6 ± 0.2	15.2 ± 0.1*	15.0 ± 0.2**
Erythrocytes (10^6/µL)	9.92 ± 0.08	9.97 ± 0.12	10.37 ± 0.12*	10.50 ± 0.11**	10.41 ± 0.12**	10.53 ± 0.14**
Reticulocytes (10^6/µL)	0.28 ± 0.03	0.26 ± 0.02	0.29 ± 0.01	0.36 ± 0.02	0.30 ± 0.01	0.30 ± 0.01
Reticulocytes (%)	2.84 ± 0.28	2.63 ± 0.17	2.78 ± 0.14	3.43 ± 0.21	2.90 ± 0.13	2.86 ± 0.11
Nucleated erythrocytes (10^3/µL)	0.22 ± 0.15	0.10 ± 0.10	0.00 ± 0.00	0.40 ± 0.22	0.00 ± 0.00	0.10 ± 0.10
Mean cell volume (fL)	46.7 ± 0.1	46.3 ± 0.3	45.8 ± 0.1**	44.9 ± 0.2**	44.0 ± 0.2**	43.3 ± 0.4**
Mean cell hemoglobin (pg)	15.8 ± 0.1	15.6 ± 0.1*	15.3 ± 0.1**	14.9 ± 0.0**	14.6 ± 0.1**	14.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.1	33.6 ± 0.3	33.3 ± 0.2	33.1 ± 0.2*	33.1 ± 0.2*	33.0 ± 0.2*
Platelets (10^3/µL)	838.2 ± 52.4	901.1 ± 50.8	799.2 ± 31.0	769.5 ± 32.8	836.2 ± 37.7	839.5 ± 34.0
Leukocytes (10^3/µL)	5.96 ± 0.80	5.20 ± 0.49	4.39 ± 0.54	5.05 ± 0.50	5.17 ± 0.45	4.63 ± 0.70
Segmented neutrophils (10^3/µL)	0.67 ± 0.09	0.52 ± 0.06	0.39 ± 0.04*	0.52 ± 0.09	0.53 ± 0.04	0.51 ± 0.07
Lymphocytes (10^3/µL)	5.03 ± 0.71	4.47 ± 0.42	3.77 ± 0.49	4.20 ± 0.44	4.37± 0.41	3.91 ± 0.61
Monocytes (10^3/µL)	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.12 ± 0.04	0.09 ± 0.01	0.07 ± 0.01
Basophils (10^3/µL)	0.024 ± 0.004	0.014 ± 0.002	0.017 ± 0.004	0.013 ± 0.003	0.019 ± 0.002	0.016 ± 0.004
Eosinophils (10^3/µL)	0.10 ± 0.02	0.10 ± 0.01	0.13 ± 0.04	0.17 ± 0.03	0.13 ± 0.03	0.10 ± 0.02
Large unstained cells (10^3/mm^3)	0.043 ± 0.007	0.034 ± 0.006	0.021 ± 0.004*	0.025 ± 0.004	0.029 ± 0.005	0.027 ± 0.011*

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

TABLE C3
Hematology and Clinical Chemistry Data for Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Hematology				
n	7	9	9	10
Hematocrit (automated) (%)	47.1 ± 0.3	47.2 ± 0.3	47.4 ± 0.4	47.4 ± 0.4
Hematocrit (manual) (%)	48.6 ± 0.8	47.9 ± 0.2	47.6 ± 0.3	47.8 ± 0.3[d]
Hemoglobin (g/dL)	15.1 ± 0.2	15.1 ± 0.1	15.0 ± 0.1	15.1 ± 0.2
Erythrocytes (10^6/µL)	9.88 ± 0.09	10.13 ± 0.09	10.20 ± 0.13	10.60 ± 0.10**
Reticulocytes (10^6/µL)	0.24 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.26 ± 0.01
Mean cell volume (fL)	47.7 ± 0.2	46.6 ± 0.2**	46.4 ± 0.2	44.7 ± 0.1
Mean cell hemoglobin (pg)	15.3 ± 0.1	14.9 ± 0.1**	14.7 ± 0.1**	14.2 ± 0.0**
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.1	32.1 ± 0.1	31.8 ± 0.1*	31.8 ± 0.1
Platelets (10^3/µL)	1,129 ± 55	1,079 ± 76	1,217 ± 49	1,053 ± 89
Leukocytes (10^3/µL)	4.54 ± 0.36	4.88 ± 0.45	4.78 ± 0.43	4.24 ± 0.45
Segmented neutrophils (10^3/µL)	0.65 ± 0.08	0.59 ± 0.06	0.68 ± 0.13	0.58 ± 0.07
Lymphocytes (10^3/µL)	3.73 ± 0.37	4.07 ± 0.39	3.93 ± 0.40	3.51 ± 0.38
Monocytes (10^3/µL)	0.10 ± 0.02	0.11 ± 0.02	0.09 ± 0.02	0.07 ± 0.01
Basophils (10^3/µL)	0.023 ± 0.007	0.033 ± 0.014	0.030 ± 0.005	0.022 ± 0.006
Eosinophils (10^3/µL)	0.04 ± 0.01	0.07 ± 0.02	0.05 ± 0.02	0.06 ± 0.02
Clinical Chemistry				
n	8	7	9	10
Urea nitrogen (mg/dL)	20.6 ± 1.5[b]	20.3 ± 0.9[c]	20.6 ± 1.1	21.9 ± 1.2[d]
Creatinine (mg/dL)	0.34 ± 0.02[e]	0.34 ± 0.02	0.32 ± 0.02	0.35 ± 0.04[f]
Total protein (g/dL)	5.7 ± 0.0[b]	5.8 ± 0.0	5.9 ± 0.0	5.9 ± 0.1[d]
Albumin (g/dL)	3.8 ± 0.0	3.9 ± 0.0	3.9 ± 0.0	3.9 ± 0.0*
Alanine aminotransferase (IU/L)	68 ± 8	68 ± 9	57 ± 4[g]	67 ± 4
Alkaline phosphatase (IU/L)	84 ± 1	82 ± 2	77 ± 2*[g]	76 ± 2**
Creatine kinase (IU/L)	150 ± 38[b]	161 ± 65	236 ± 48	226 ± 29[d]
Sorbitol dehydrogenase (IU/L)	41 ± 4[d]	39 ± 4[f]	29 ± 1**[g]	27 ± 1**
Bile acids (µmol/L)	29.0 ± 1.3[d]	27.3 ± 1.0[f]	25.7 ± 0.9[g]	25.0 ± 1.0*

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=7
[c] n=6
[d] n=9
[e] n=5
[f] n=8
[g] n=10

TABLE C4
Hematology and Clinical Chemistry Data for Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Hematology				
n	10	10	10	10
Hematocrit (automated) (%)	48.9 ± 0.3	48.4 ± 0.2	48.0 ± 0.3	48.8 ± 0.6
Hematocrit (manual) (%)	49.9 ± 0.5	49.3 ± 0.3	49.2 ± 0.3	49.5 ± 0.5
Hemoglobin (g/dL)	16.3 ± 0.1	16.0 ± 0.1	15.9 ± 0.1*	16.1 ± 0.2
Erythrocytes (10^6/µL)	10.91 ± 0.06	11.04 ± 0.06	11.18 ± 0.10*	11.47 ± 0.13**
Reticulocytes (10^6/µL)	0.25 ± 0.01	0.25 ± 0.00	0.25 ± 0.01	0.25 ± 0.01
Mean cell volume (fL)	44.8 ± 0.2	43.8 ± 0.2**	42.9 ± 0.2	42.6 ± 0.2
Mean cell hemoglobin (pg)	15.0 ± 0.1	14.5 ± 0.1**	14.2 ± 0.1**	14.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	33.4 ± 0.1	33.1 ± 0.2	33.1 ± 0.1	33.0 ± 0.2
Platelets (10^3/µL)	1,200 ± 31	1,202 ± 42	1,284 ± 49	1,280 ± 56
Leukocytes (10^3/µL)	7.15 ± 066	6.00 ± 0.50	8.10 ± 0.67	8.26 ± 0.72
Segmented neutrophils (10^3/µL)	1.11 ± 0.10	0.97 ± 0.07	1.24 ± 0.11	1.42 ± 0.10
Lymphocytes (10^3/µL)	5.88 ± 0.55	4.90 ± 0.47	6.63 ± 0.61	6.61 ± 0.60
Monocytes (10^3/µL)	0.13 ± 0.04	0.10 ± 0.04	0.15 ± 0.03	0.16 ± 0.04
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.03 ± 0.01	0.02 ± 0.01	0.08 ± 0.03	0.08 ± 0.02
Clinical Chemistry				
n	10	8	8	9
Urea nitrogen (mg/dL)	21.0 ± 0.8[b]	21.2 ± 1.1[b]	19.8 ± 1.0[c]	22.0 ± 0.8[b]
Creatinine (mg/dL)	0.30 ± 0.00[d]	0.28 ± 0.03[e]	0.30 ± 0.00[d]	0.30 ± 0.00[e]
Total protein (g/dL)	6.1 ± 0.1[f]	6.1 ± 0.1[b]	5.7 ± 0.1*[g]	5.4 ± 0.1**[c]
Albumin (g/dL)	3.9 ± 0.1[g]	3.9 ± 0.0	3.7 ± 0.0*[g]	3.7 ± 0.0**[g]
Alanine aminotransferase (IU/L)	41 ± 2[h]	43 ± 3	55 ± 3**	54 ± 4**[g]
Alkaline phosphatase (IU/L)	85 ± 2[h]	89 ± 1	87 ± 2	83 ± 2
Creatine kinase (IU/L)	154 ± 40[c]	201 ± 43[g]	165 ± 18[c]	205 ± 52[g]
Sorbitol dehydrogenase (IU/L)	24 ± 1	24 ± 1	22 ± 1[i]	21 ± 0
Bile acids (µmol/L)	24.1 ± 0.4	24.3 ± 1.1	22.3 ± 1.3[h]	22.9 ± 0.7

* Significantly different ($P \le 0.05$) from the control group by Dunn's or Shirley's test
** $P \le 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=5
[c] n=6
[d] n=2
[e] n=4
[f] n=8
[g] n=7
[h] n=9
[i] n=10

TABLE C5
Hematology and Clinical Chemistry Data for Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
Hematology				
n	10	10	10	9
Hematocrit (automated) (%)	47.6 ± 0.3	47.6 ± 0.5	46.4 ± 0.3	42.8 ± 0.5
Hematocrit (manual) (%)	48.2 ± 0.3	48.2 ± 0.6	47.5 ± 0.3	46.6 ± 0.6*
Hemoglobin (g/dL)	15.0 ± 0.1	15.1 ± 0.2	14.7 ± 0.1	14.2 ± 0.1**
Erythrocytes (10^6/µL)	10.40 ± 0.08	10.77 ± 0.21	10.63 ± 0.08	10.56 ± 0.13
Reticulocytes (10^6/µL)	0.29 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.31 ± 0.01
Mean cell volume (fL)	45.8 ± 0.2	44.2 ± 0.4	43.7 ± 0.3**	40.5 ± 0.3
Mean cell hemoglobin (pg)	14.4 ± 0.1	14.1 ± 0.1**	13.8 ± 0.1**	13.5 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	31.4 ± 0.1	31.8 ± 0.2	31.7 ± 0.1	33.2 ± 0.3
Platelets (10^3/µL)	1,343 ± 60	1,382 ± 46	1,435 ± 62	1,569 ± 69*
Leukocytes (10^3/µL)	8.48 ± 0.74	6.18 ± 0.75	8.09 ± 0.98	8.08 ± 1.01
Segmented neutrophils (10^3/µL)	0.62 ± 0.06	0.44 ± 0.05	0.52 ± 0.05	0.58 ± 0.06
Lymphocytes (10^3/µL)	7.57 ± 0.70	5.53 ± 0.69	7.31 ± 0.90	7.25 ± 0.97
Monocytes (10^3/µL)	0.11 ± 0.01	0.08 ± 0.01	0.10 ± 0.02	0.13 ± 0.02
Basophils (10^3/µL)	0.057 ± 0.005	0.036 ± 0.004	0.054 ± 0.008	0.044 ± 0.011
Eosinophils (10^3/µL)	0.13 ± 0.02	0.09 ± 0.02	0.11 ± 0.03	0.09 ± 0.03
Clinical Chemistry				
n	10	6	9	7
Urea nitrogen (mg/dL)	24.4 ± 1.1[b]	23.7 ± 0.3[c]	24.7 ± 1.2[c]	29.7 ± 7.2[c]
Creatinine (mg/dL)	0.32 ± 0.02[d]	0.30 ± 0.00[c]	0.33 ± 0.03[c]	0.30[e]
Total protein (g/dL)	5.7 ± 0.0[b]	5.7 ± 0.1[d]	5.8 ± 0.1[f]	5.6 ± 0.1[c]
Albumin (g/dL)	3.9 ± 0.0[g]	3.9 ± 0.0	4.0 ± 0.0[d]	3.9 ± 0.0[h]
Alanine aminotransferase (IU/L)	38 ± 2	38 ± 2	44 ± 3[b]	73 ± 4**
Alkaline phosphatase (IU/L)	73 ± 1	72 ± 2	70 ± 2[b]	79 ± 4
Creatine kinase (IU/L)	222 ± 27[g]	216 ± 50	301 ± 107[d]	207 ± 40[f]
Sorbitol dehydrogenase (IU/L)	31 ± 1	24 ± 1**[g]	25 ± 2*	29 ± 1[g]
Bile acids (µmol/L)	24.7 ± 0.5	23.4 ± 0.7[g]	23.3 ± 0.6	25.4 ± 0.7[b]

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=8
[c] n=3
[d] n=5
[e] n=1
[f] n=4
[g] n=9
[h] n=6

APPENDIX D
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE D1	Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)	D-2
TABLE D2	Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F_1 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)	D-4
TABLE D3	Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F_1 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)	D-5
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TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	330 ± 8	322 ± 4	337 ± 6	330 ± 4	316 ± 3	298 ± 5**
Heart						
Absolute	0.862 ± 0.021	0.771 ± 0.028*	0.850 ± 0.020	0.867 ± 0.027	0.797 ± 0.010	0.836 ± 0.022
Relative	2.610 ± 0.024	2.392 ± 0.082*	2.526 ± 0.037	2.629 ± 0.070	2.526 ± 0.033	2.805 ± 0.058
R. Kidney						
Absolute	1.025 ± 0.025	1.014 ± 0.022	1.089 ± 0.025	1.022 ± 0.017	0.984 ± 0.011	0.964 ± 0.019
Relative	3.105 ± 0.038	3.145 ± 0.055	3.239 ± 0.066	3.103 ± 0.048	3.117 ± 0.026	3.234 ± 0.025
Liver						
Absolute	10.894 ± 0.417	10.297 ± 0.278	11.449 ± 0.377	10.508 ± 0.178	9.197 ± 0.166**	8.881 ± 0.184**
Relative	32.911 ± 0.650	31.911 ± 0.608	33.981 ± 0.749	31.904 ± 0.539	29.145 ± 0.533**	29.799 ± 0.351**
Lung						
Absolute	1.348 ± 0.045	1.280 ± 0.032	1.441 ± 0.053	1.321 ± 0.045	1.223 ± 0.033[b]	1.191 ± 0.035*
Relative	4.077 ± 0.071	3.968 ± 0.072	4.276 ± 0.114	4.004 ± 0.115	3.857 ± 0.109[b]	3.998 ± 0.102
Spleen						
Absolute	0.639 ± 0.018	0.596 ± 0.014	0.616 ± 0.015	0.596 ± 0.019	0.533 ± 0.008**	0.597 ± 0.012**
Relative	1.935 ± 0.031	1.848 ± 0.030	1.832 ± 0.036	1.806 ± 0.045*	1.689 ± 0.022**	2.004 ± 0.025
R. Testis						
Absolute	1.321 ± 0.036	1.342 ± 0.034	1.367 ± 0.045	1.369 ± 0.014	1.236 ± 0.038	1.352 ± 0.026
Relative	4.009 ± 0.108	4.158 ± 0.077	4.066 ± 0.120	4.157 ± 0.031	3.917 ± 0.122	4.540 ± 0.063**
Thymus						
Absolute	0.231 ± 0.008	0.224 ± 0.016	0.244 ± 0.013	0.248 ± 0.015	0.196 ± 0.006	0.217 ± 0.011
Relative	0.701 ± 0.029	0.694 ± 0.045	0.723 ± 0.031	0.750 ± 0.043	0.622 ± 0.021	0.727 ± 0.033

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Female						
Necropsy body wt	193 ± 3	215 ± 3	199 ± 2	196 ± 2	193 ± 3	185 ± 2
Heart						
Absolute	0.573 ± 0.012	0.607 ± 0.006	0.587 ± 0.011	0.570 ± 0.015	0.570 ± 0.013	0.563 ± 0.014
Relative	2.973 ± 0.036	2.829 ± 0.041	2.946 ± 0.048	2.872 ± 0.071	2.954 ± 0.038	3.045 ± 0.057
R. Kidney						
Absolute	0.643 ± 0.019	0.712 ± 0.011*	0.708 ± 0.012	0.705 ± 0.016	0.692 ± 0.026	0.671 ± 0.019
Relative	3.339 ± 0.088	3.315 ± 0.035	3.553 ± 0.046	3.552 ± 0.073	3.582 ± 0.102*	3.630 ± 0.087*
Liver						
Absolute	5.751 ± 0.130	6.028 ± 0.157	5.954 ± 0.145	5.978 ± 0.121	5.623 ± 0.195	5.599 ± 0.176
Relative	29.839 ± 0.421	28.076 ± 0.728	29.880 ± 0.661	30.117 ± 0.503	29.130 ± 0.791	30.249 ± 0.688
Lung						
Absolute	0.957 ± 0.037	0.979 ± 0.019	1.007 ± 0.035	0.947 ± 0.043	0.957 ± 0.017	0.858 ± 0.015
Relative	4.961 ± 0.153	4.561 ± 0.094	5.058 ± 0.190	4.767 ± 0.199	4.968 ± 0.094	4.645 ± 0.081
Spleen						
Absolute	0.408 ± 0.010	0.438 ± 0.009	0.431 ± 0.009	0.441 ± 0.007	0.435 ± 0.014	0.441 ± 0.005
Relative	2.120 ± 0.053	2.038 ± 0.027	2.164 ± 0.048	2.222 ± 0.026	2.253 ± 0.051*	2.387 ± 0.027**
Thymus						
Absolute	0.209 ± 0.009	0.216 ± 0.009	0.230 ± 0.007	0.219 ± 0.007	0.210 ± 0.007	0.213 ± 0.006
Relative	1.081 ± 0.040	1.005 ± 0.039	1.154 ± 0.028	1.102 ± 0.029	1.088 ± 0.033	1.155 ± 0.035

* 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

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 1)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	38.9 ± 1.1	36.9 ± 0.9	35.6 ± 1.0*	33.3 ± 0.8**	31.1 ± 1.1**	31.2 ± 0.5**
Heart						
Absolute	0.148 ± 0.003	0.152 ± 0.004	0.154 ± 0.004	0.142 ± 0.006	0.141 ± 0.005	0.141 ± 0.005
Relative	3.823 ± 0.111	4.119 ± 0.066	4.356 ± 0.149*	4.271 ± 0.143*	4.540 ± 0.065**	4.542 ± 0.199**
R. Kidney						
Absolute	0.282 ± 0.008	0.282 ± 0.007	0.262 ± 0.008	0.257 ± 0.006*	0.240 ± 0.007**	0.255 ± 0.005**
Relative	7.251 ± 0.106	7.678 ± 0.292	7.429 ± 0.347	7.752 ± 0.199	7.762 ± 0.299	8.180 ± 0.074**
Liver						
Absolute	1.599 ± 0.081	1.544 ± 0.052	1.488 ± 0.052	1.403 ± 0.046*	1.330 ± 0.060**	1.339 ± 0.038**
Relative	40.931 ± 1.219	42.012 ± 1.683	41.864 ± 0.999	42.354 ± 1.577	42.777 ± 1.306	42.936 ± 0.912
Lung						
Absolute	0.244 ± 0.018	0.198 ± 0.014	0.256 ± 0.015	0.240 ± 0.019	0.222 ± 0.015	0.224 ± 0.015
Relative	6.268 ± 0.437	5.419 ± 0.461	7.254 ± 0.490	7.258 ± 0.596	7.171 ± 0.497	7.174 ± 0.459
Spleen						
Absolute	0.068 ± 0.003	0.067 ± 0.002	0.064 ± 0.002	0.064 ± 0.003	0.063 ± 0.002	0.060 ± 0.002
Relative	1.745 ± 0.050	1.820 ± 0.048	1.823 ± 0.105	1.930 ± 0.096	2.033 ± 0.059*	1.928 ± 0.070*
R. Testis						
Absolute	0.116 ± 0.001	0.118 ± 0.003	0.123 ± 0.003	0.117 ± 0.002	0.115 ± 0.005	0.115 ± 0.002
Relative	3.003 ± 0.054	3.209 ± 0.102	3.500 ± 0.187**	3.524 ± 0.054**	3.703 ± 0.164**	3.681 ± 0.079**
Thymus						
Absolute	0.033 ± 0.001	0.033 ± 0.001	0.034 ± 0.001	0.030 ± 0.001	0.031 ± 0.001	0.031 ± 0.001
Relative	0.849 ± 0.028	0.899 ± 0.034	0.953 ± 0.017	0.897 ± 0.030	0.996 ± 0.031**	1.002 ± 0.039**
Female						
Necropsy body wt	28.2 ± 0.7	28.6 ± 0.8	26.3 ± 0.7	27.1 ± 0.8	25.1 ± 0.3**	24.8 ± 0.4**
Heart						
Absolute	0.122 ± 0.003	0.117 ± 0.009	0.123 ± 0.003	0.127 ± 0.006	0.120 ± 0.003	0.111 ± 0.003
Relative	4.340 ± 0.093	4.106 ± 0.327	4.694 ± 0.128	4.708 ± 0.197	4.794 ± 0.105	4.488 ± 0.135
R. Kidney						
Absolute	0.159 ± 0.004	0.160 ± 0.003	0.154 ± 0.004	0.161 ± 0.004	0.154 ± 0.004	0.150 ± 0.003
Relative	5.658 ± 0.135	5.609 ± 0.130	5.872 ± 0.132	5.984 ± 0.187	6.159 ± 0.185	6.070 ± 0.151
Liver						
Absolute	1.151 ± 0.031	1.139 ± 0.042	1.057 ± 0.024	1.109 ± 0.037	1.041 ± 0.023*	0.990 ± 0.019**
Relative	40.964 ± 0.983	39.748 ± 0.713	40.321 ± 0.877	41.027 ± 0.737	41.538 ± 0.706	40.010 ± 0.783
Lung						
Absolute	0.206 ± 0.008	0.227 ± 0.016	0.244 ± 0.012	0.234 ± 0.016	0.217 ± 0.008	0.203 ± 0.012
Relative	7.322 ± 0.248	7.998 ± 0.639	9.302 ± 0.412*	8.670 ± 0.547	8.653 ± 0.278	8.183 ± 0.435
Spleen						
Absolute	0.074 ± 0.002	0.080 ± 0.002	0.080 ± 0.002	0.081 ± 0.004	0.071 ± 0.003	0.074 ± 0.003
Relative	2.631 ± 0.064	2.811 ± 0.102	3.061 ± 0.109*	3.002 ± 0.142	2.833 ± 0.120	2.989 ± 0.103
Thymus						
Absolute	0.035 ± 0.002	0.040 ± 0.002	0.040 ± 0.001	0.044 ± 0.002**	0.035 ± 0.002	0.039 ± 0.002
Relative	1.234 ± 0.075	1.385 ± 0.067	1.519 ± 0.029**	1.618 ± 0.058**	1.381 ± 0.071**	1.572 ± 0.066**

* 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).

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	10	10	10	10
Necropsy body wt	46.8 ± 0.8	46.7 ± 0.6	42.7 ± 1.0**	36.4 ± 0.5**
Heart				
Absolute	0.190 ± 0.004	0.197 ± 0.006	0.185 ± 0.004	0.180 ± 0.004
Relative	4.062 ± 0.092	4.217 ± 0.132	4.352 ± 0.128	4.933 ± 0.087**
R. Kidney				
Absolute	0.325 ± 0.010	0.315 ± 0.008	0.298 ± 0.007*	0.263 ± 0.004**
Relative	6.938 ± 0.195	6.743 ± 0.155	7.014 ± 0.221	7.225 ± 0.104
Lung				
Absolute	0.216 ± 0.006	0.203 ± 0.009	0.197 ± 0.009	0.186 ± 0.004**
Relative	4.621 ± 0.137	4.346 ± 0.183	4.635 ± 0.219	5.129 ± 0.130
Spleen				
Absolute	0.085 ± 0.004	0.079 ± 0.003	0.078 ± 0.003	0.068 ± 0.002**
Relative	1.801 ± 0.060	1.694 ± 0.050	1.833 ± 0.081	1.870 ± 0.049
R. Testis				
Absolute	0.127 ± 0.003	0.126 ± 0.002	0.126 ± 0.002	0.125 ± 0.002
Relative	2.730 ± 0.079	2.697 ± 0.048	2.976 ± 0.094*	3.437 ± 0.093**
Thymus				
Absolute	0.067 ± 0.004	0.060 ± 0.007	0.060 ± 0.004	0.049 ± 0.003*
Relative	1.424 ± 0.083	1.280 ± 0.129	1.412 ± 0.109	1.333 ± 0.084

* 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 D4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	10	10	10	10
Necropsy body wt	28.8 ± 0.5	28.2 ± 0.6	27.0 ± 0.3**	25.7 ± 0.4**
Heart				
Absolute	0.193 ± 0.008	0.181 ± 0.005	0.172 ± 0.005*	0.169 ± 0.006*
Relative	6.674 ± 0.242	6.407 ± 0.173	6.368 ± 0.193	6.564 ± 0.167
R. Kidney				
Absolute	0.249 ± 0.006	0.253 ± 0.004	0.225 ± 0.005**	0.221 ± 0.005**
Relative	8.635 ± 0.148	8.974 ± 0.144	8.360 ± 0.159	8.607 ± 0.099
Liver				
Absolute	1.599 ± 0.030	1.620 ± 0.053	1.509 ± 0.019	1.386 ± 0.029**
Relative	55.443 ± 0.770	57.300 ± 1.078	56.042 ± 0.751	53.937 ± 0.759
Lung				
Absolute	0.219 ± 0.012	0.186 ± 0.008	0.198 ± 0.008	0.194 ± 0.012
Relative	7.552 ± 0.311	6.629 ± 0.325	7.343 ± 0.292	7.556 ± 0.435
Spleen				
Absolute	0.089 ± 0.003	0.085 ± 0.003	0.083 ± 0.002	0.081 ± 0.002
Relative	3.097 ± 0.082	3.027 ± 0.114	3.070 ± 0.103	3.149 ± 0.079
R. Testis				
Absolute	0.100 ± 0.002	0.099 ± 0.002	0.096 ± 0.002	0.096 ± 0.003
Relative	3.468 ± 0.097	3.506 ± 0.088	3.565 ± 0.048	3.744 ± 0.115
Thymus				
Absolute	0.039 ± 0.001	0.043 ± 0.004	0.041 ± 0.002	0.039 ± 0.002
Relative	1.352 ± 0.050	1.508 ± 0.103	1.518 ± 0.084	1.514 ± 0.107

* 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 D5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	5	5	5	5
Necropsy body wt	42.6 ± 1.5	36.0 ± 2.6*	35.4 ± 1.3**	27.4 ± 0.2**
Heart				
Absolute	0.196 ± 0.011	0.194 ± 0.013	0.187 ± 0.008	0.157 ± 0.007*
Relative	4.644 ± 0.391	5.448 ± 0.394	5.317 ± 0.363	5.739 ± 0.268
R. Kidney				
Absolute	0.210 ± 0.002	0.237 ± 0.028	0.219 ± 0.005	0.185 ± 0.004
Relative	4.960 ± 0.202	6.569 ± 0.598**	6.203 ± 0.154**	6.763 ± 0.131**
Liver				
Absolute	1.801 ± 0.077	1.585 ± 0.165	1.516 ± 0.054	1.241 ± 0.036**
Relative	42.235 ± 0.806	43.669 ± 1.310	42.830 ± 0.588	45.354 ± 1.172
Lung				
Absolute	0.194 ± 0.009	0.188 ± 0.014	0.177 ± 0.005	0.174 ± 0.006
Relative	4.560 ± 0.173	5.218 ± 0.213	5.012 ± 0.123	6.349 ± 0.237**
Spleen				
Absolute	0.082 ± 0.004	0.076 ± 0.006	0.079 ± 0.004	0.064 ± 0.002*
Relative	1.936 ± 0.108	2.114 ± 0.121	2.227 ± 0.075	2.347 ± 0.070**
R. Testis				
Absolute	0.115 ± 0.002	0.112 ± 0.004	0.118 ± 0.003	0.109 ± 0.006
Relative	2.708 ± 0.107	3.138 ± 0.125	3.349 ± 0.179*	3.992 ± 0.216**
Thymus				
Absolute	0.062 ± 0.003	0.053 ± 0.003	0.055 ± 0.003	0.042 ± 0.003**
Relative	1.467 ± 0.073	1.482 ± 0.064	1.544 ± 0.084	1.550 ± 0.097

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

APPENDIX E
REPRODUCTIVE TISSUE EVALUATIONS

TABLE E1	Summary of Reproductive Tissue Evaluations for Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)	E-2
TABLE E2	Summary of Reproductive Tissue Evaluations for Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)	E-3
TABLE E3	Summary of Reproductive Tissue Evaluations for Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)	E-4

TABLE E1
Summary of Reproductive Tissue Evaluations for Male B6C3F$_1$ Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	46.8 ± 0.8	46.7 ± 0.6	42.7 ± 1.0**	36.4 ± 0.5**
L. Cauda epididymis	0.0142 ± 0.0005	0.0140 ± 0.0005	0.0150 ± 0.0004	0.0146 ± 0.0005
L. Epididymis	0.0457 ± 0.0008	0.0446 ± 0.0010	0.0439 ± 0.0006	0.0430 ± 0.0009
L. Testis	0.1192 ± 0.0020	0.1230 ± 0.0016	0.1176 ± 0.0010	0.1183 ± 0.0016
Spermatid measurements				
Spermatid heads (10^7/g testis)	19.52 ± 0.42	20.31 ± 0.68	20.08 ± 0.62	20.72 ± 0.33
Spermatid heads (10^7/testis)	2.19 ± 0.07	2.33 ± 0.07	2.19 ± 0.07	2.32 ± 0.05
Epididymal spermatozoal measurements				
Sperm heads (10^7/g cauda epididymis)	87.02 ± 8.41	103.26 ± 6.69	84.04 ± 7.05	102.93 ± 11.18
Sperm heads (10^7/cauda epididymis)	1.21 ± 0.09	1.45 ± 0.11	1.26 ± 0.11	1.48 ± 0.14
Sperm motility (%)	87.67 ± 0.89	88.05 ± 0.65	88.61 ± 0.83	87.63 ± 1.19

** 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 E2
Summary of Reproductive Tissue Evaluations for Male BALB/c Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	28.8 ± 0.5	28.2 ± 0.6	27.0 ± 0.3**	25.7 ± 0.4**
L. Cauda epididymis	0.0101 ± 0.0006	0.0109 ± 0.0003	0.0097 ± 0.0004	0.0101 ± 0.0005
L. Epididymis	0.0351 ± 0.0006	0.0355 ± 0.0009	0.0349 ± 0.0006	0.0333 ± 0.0007
L. Testis	0.0973 ± 0.0029	0.0962 ± 0.0021	0.0928 ± 0.0017	0.0929 ± 0.0022
Spermatid measurements				
Spermatid heads (10^7/g testis)	14.07 ± 1.05	14.90 ± 0.45	14.72 ± 0.29	15.87 ± 0.57
Spermatid heads (10^7/testis)	1.29 ± 0.12	1.35 ± 0.06	1.31 ± 0.04	1.35 ± 0.06
Epididymal spermatozoal measurements				
Sperm heads (10^7/g cauda epididymis)	111.70 ± 7.40	110.40 ± 5.50	120.70 ± 5.70	114.90 ± 11.60
Sperm heads (10^7/cauda epididymis)	1.10 ± 0.03	1.20 ± 0.05	1.17 ± 0.07	1.15 ± 0.11
Sperm motility (%)	91.42 ± 0.40	89.75 ± 0.37*	90.54 ± 0.43	91.28 ± 0.46

* Significantly different ($P \leq 0.05$) from the control group by 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 and sperm head measurements).

TABLE E3
Summary of Reproductive Tissue Evaluations for Male *am3*-C57BL/6 Mice in the 3-Month Drinking Water Study of Sodium Dichromate Dihydrate (Study 2)[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L
n	5	5	5	5
Weights (g)				
Necropsy body wt	42.6 ± 1.5	36.0 ± 2.6*	35.4 ± 1.3**	27.4 ± 0.2**
L. Cauda epididymis	0.0131 ± 0.0003	0.0114 ± 0.0007	0.0175 ± 0.0040	0.0119 ± 0.0009
L. Epididymis	0.0426 ± 0.0021	0.0387 ± 0.0016	0.0471 ± 0.0062	0.0429 ± 0.0053
L. Testis	0.1124 ± 0.0019	0.1061 ± 0.0038	0.1098 ± 0.0024	0.0996 ± 0.0015**
Spermatid measurements				
Spermatid heads (10^7/g testis)	20.91 ± 1.41	19.55 ± 0.33	19.60 ± 0.65	20.04 ± 0.72
Spermatid heads (10^7/testis)	2.24 ± 0.16	2.01 ± 0.09	2.06 ± 0.03	1.93 ± 0.08
Epididymal spermatozoal measurements				
Sperm heads (10^7/g cauda epididymis)	123.00 ± 11.20	114.20 ± 9.80	108.70 ± 18.10	123.30 ± 8.40
Sperm heads (10^7/cauda epididymis)	1.61± 0.15	1.30 ± 0.14	1.66 ± 0.15	1.47 ± 0.16
Sperm motility (%)	89.90 ± 0.53	90.28 ± 0.17	90.22 ± 0.92[b]	90.10 ± 0.59[b]

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** Significantly different ($P \leq 0.01$) from the control group by Williams' test (body weight) or Dunnett's test (left testis weight)
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (left cauda epididymal and epididymal weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).
[b] n=4

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF SODIUM DICHROMATE DIHYDRATE F-2
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS F-3
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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF SODIUM DICHROMATE DIHYDRATE

Sodium dichromate dihydrate was obtained from Aldrich Chemical Company (Milwaukee, WI) in two lots (15301BI and 13822LI). The two lots were combined at the analytical chemistry laboratory, Battelle Memorial Institute (Columbus, OH), and assigned a new lot number (062001). Lot 062001 was used in the 3-month studies in F344/N rats and B6C3F$_1$ mice (study 1). An additional shipment of lot 13822LI was obtained from Aldrich Chemical Company and used in the 3-month studies in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 mice (study 2). Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory (lots 062001 and 13822LI) and by the study laboratories at Southern Research Institute (Birmingham, AL; lot 062001) and Battelle Columbus Operations (Columbus, OH; lot 13822LI). Karl Fischer titration (lots 062001 and 13822LI) and elemental analysis using inductively coupled plasma-atomic emission spectroscopy (ICP-AES; lot 062001) were performed by Galbraith Laboratories, Inc. (Knoxville, TN); elemental analysis using ICP-AES (lot 13822LI) was performed by Battelle Northwest Operations (Richland, WA); and elemental analysis using proton-induced X-ray emission spectroscopy (PIXE; lots 062001 and 13822LI) was performed by Elemental Analysis Corporation (Lexington, KY). Reports on analyses performed in support of the sodium dichromate dihydrate studies are on file at the National Institute of Environmental Health Sciences.

Lot 062001, an orange crystalline solid, was identified as sodium dichromate dihydrate by the analytical chemistry laboratory using X-ray diffraction (XRD), by the analytical chemistry laboratory and Galbraith Laboratories, Inc., using elemental analysis by ICP-AES, and by Elemental Analysis Corporation using elemental analysis by PIXE. Lot 13822LI, an orange crystalline solid, was identified as sodium dichromate dihydrate by the analytical chemistry laboratory using XRD, by the analytical chemistry laboratory and Battelle Northwest Operations using elemental analysis by ICP-AES, and by Elemental Analysis Corporation using elemental analysis by PIXE. The XRD powder patterns were consistent with a reference pattern (International Centre for Diffraction Data, 2000); XRD diffractograms are presented in Figure F1. Elemental analyses for chromium and sodium were in agreement with the theoretical values for sodium dichromate dihydrate, and PIXE indicated the absence of significant metallic impurities. A PIXE X-ray spectrum is presented in Figure F2.

The moisture content of lots 062001 and 13822LI was determined by Karl Fischer titration and, for lot 13822LI, weight loss on drying was performed by the analytical chemistry laboratory. The purity of lot 062001 was determined by the analytical chemistry laboratory using differential scanning calorimetry (DSC), titration of the dichromate ion with sodium thiosulfate and potassium ferrocyanide, and speciation of the chromium ions using liquid chromatography-inductively coupled plasma-mass spectrometry (LC-ICP-MS) and by the study laboratory using potentiometric titrimetric analysis. The purity of lot 13822LI was determined by the analytical chemistry laboratory using DSC, titration of the dichromate ion with sodium thiosulfate and potassium ferrocyanide, and LC-ICP-MS and by the study laboratory using titration with sodium thiosulfate. DSC was performed using a Perkin-Elmer DSC-7 scanning calorimeter from 342° to 365° C (lot 062001) and from 342° to 360° C (lot 13822LI) with a temperature increase of 1° C per minute under a nitrogen atmosphere. Titration with sodium thiosulfate was performed by titrating a solution of sodium dichromate dihydrate and potassium iodide in dilute hydrochloric acid with 0.1 N sodium thiosulfate using a double sheet platinum electrode and a Brinkman Metrohm titrator. Titration with potassium ferrocyanide was performed by titrating a solution of sodium dichromate dihydrate with 0.1 N potassium ferrocyanide trihydrate as previously described. LC-ICP-MS was conducted by the analytical chemistry laboratory using system A. Potentiometric titration was performed.

A) Hewlett-Packard (Palo Alto, CA) 1100 series high performance liquid chromatography system, a Dionex Corp. (Sunnyvale, CA), IonPac AS7 ion exchange column (250 mm × 4 mm), with an isocratic mobile phase of 35 mM ammonium sulfate adjusted to pH 9.2 with ammonium hydroxide at a flow rate of 1 mL/minute and ICP-MS detection.

For lot 062001, Karl Fischer titration indicated a moisture content of 11.62%, which is in agreement with the theoretical value of 12.09%. DSC indicated a purity of 99.73% ± 0.15%. Titration with sodium thiosulfate by the analytical chemistry laboratory indicated a purity of 99.7% ± 0.1%. Titration with sodium thiosulfate by the study laboratory indicated purities of 101% and 102% relative to a frozen reference standard of the same lot. Titration with potassium ferrocyanide indicated a purity of 103.1% ± 0.2%. LC-ICP-MS indicated that the concentration of Cr III, if present, was less than 0.1%. Representative LC-ICP-MS spectra are presented in Figure F3. The overall purity of lot 062001 was determined to be greater than 99.7%.

For lot 13822LI, Karl Fischer titration indicated a moisture content of 9.16%, less than the theoretical value of 12.09%; however, the percentage weight loss on drying agreed well with the theoretical value. DSC indicated a purity of 99.10% ± 0.27%. Titration with sodium thiosulfate and potassium ferrocyanide at the analytical chemistry laboratory indicated purities of 99.1% ± 1.2% and 99.6% ± 1.6%, respectively. Titration with sodium thiosulfate at the study laboratory indicated a purity of 101.8%. LC-ICP-MS indicated that the concentration of Cr III, if present, was less than 0.1%. The overall purity of lot 13822LI was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at room temperature, protected from light in amber glass bottles (lot 062001) or in a white plastic bottle (lot 13822LI). During the studies, stability of the bulk chemical was monitored by the study laboratories using potentiometric titration (lot 062001) or titration of the dichromate ion with sodium thiosulfate (lot 13822LI). No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared four times during the 3-month studies in F344/N rats and B6C3F$_1$ mice (study 1) and five times during the 3-month studies in male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 transgenic mice (study 2) (Tables F1 and F2). Formulations used in study 1 were stored in NALGENE® containers at room temperature and protected from light. Formulations used in study 2 were stored in NALGENE® containers and refrigerated at approximately 5° C.

Stability studies of a 41.8 µg/mL sodium dichromate dihydrate dose formulation were performed by the analytical chemistry laboratory using ion chromatography (IC) by system B. Stability was confirmed for at least 42 days for dose formulations stored in sealed NALGENE® containers, protected from light, at temperatures up to room temperature and for at least 7 days when stored in drinking water bottles under simulated animal room conditions.

B) Dionex Corp. (Sunnyvale, CA), an IonPac AS7 ion exchange column (250 mm × 4 mm), using an isocratic mobile phase of 250 mM ammonium sulfate and 100 mM ammonium hydroxide at a flow rate of 1 mL/minute, with postcolumn derivitization using 2 mM 1,5-diphenylcarbazide in methanol:water:concentrated sulfuric acid (100:872:28) at a flow rate of 0.5 mL/minute and visible light (520 nm) detection.

Periodic analyses of the dose formulations of sodium dichromate dihydrate were conducted during study 1 by the study laboratory using ultraviolet spectroscopy and during study 2 by the analytical chemistry laboratory using IC by system B. During study 1, the dose formulations were analyzed three times. All 15 of the dose formulations for rats and mice were within 10% of the target concentrations (Table F3). Animal room samples and unused carboy storage samples of these dose formulations were also analyzed; 14 of 15 animal room samples for rats and all 15 of the animal room samples for mice were within 10% of target concentrations. All 15 of the unused carboy samples were within 10% of the target concentrations. During study 2, the dose formulations were analyzed three times. All nine of the dose formulations for mice were within 10% of the target concentrations (Table F4). Animal room samples and unused carboy storage samples of these dose formulations were also analyzed; all nine of the animal room samples and all nine of the carboy samples were within 10% of the target concentrations.

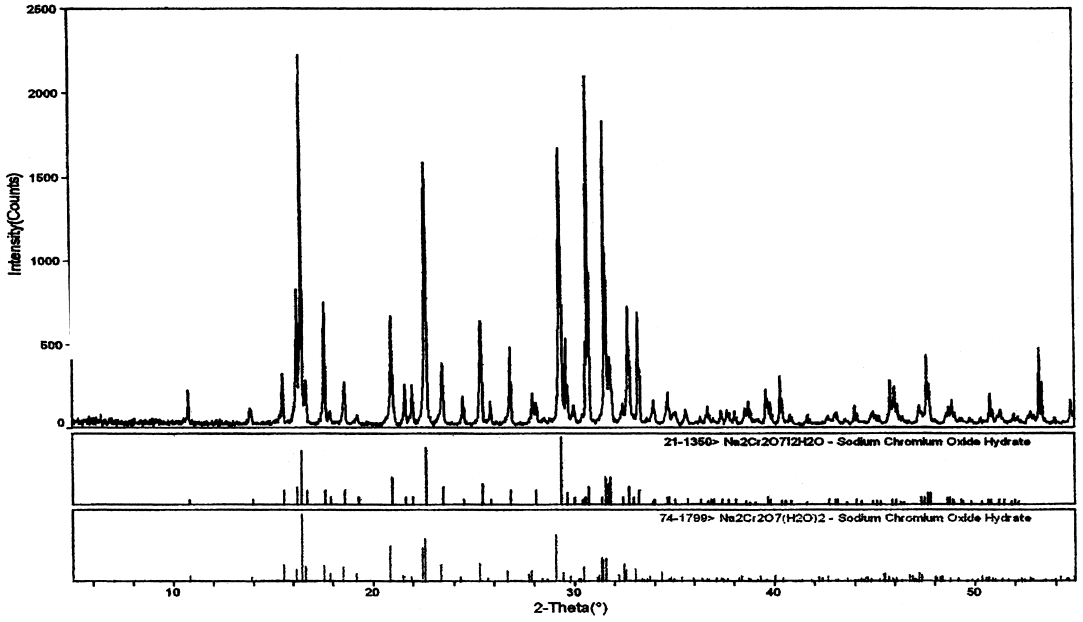

FIGURE F1
X-Ray Diffractograms of Sodium Dichromate Dihydrate (Top) and Reference Data (Center and Bottom)

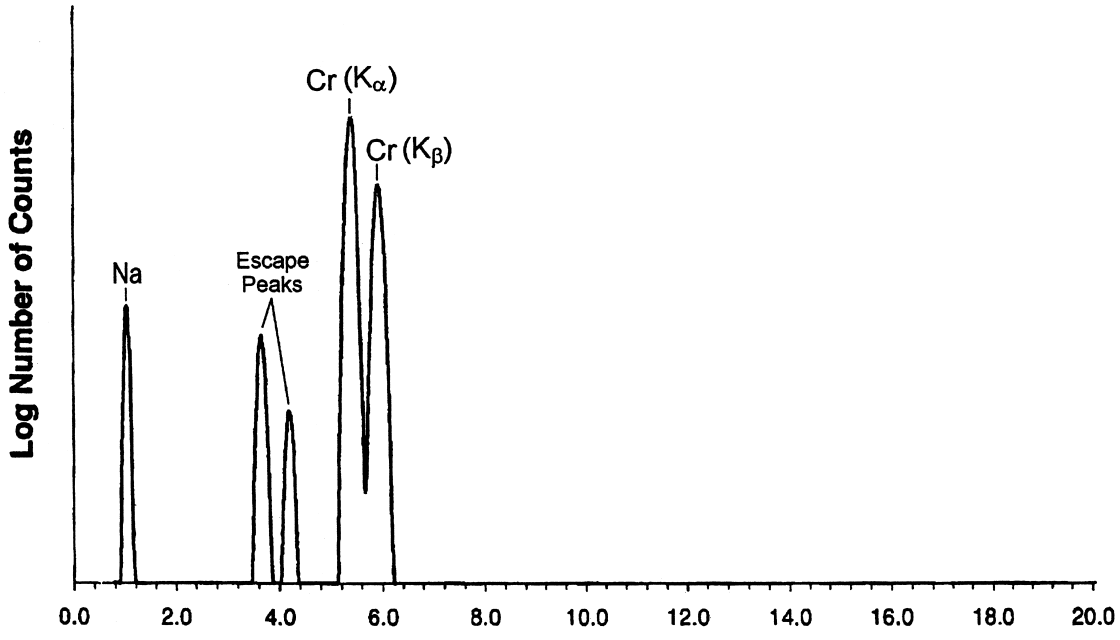

FIGURE F2
Proton Induced X-Ray Emission Spectrum of Sodium Dichromate Dihydrate

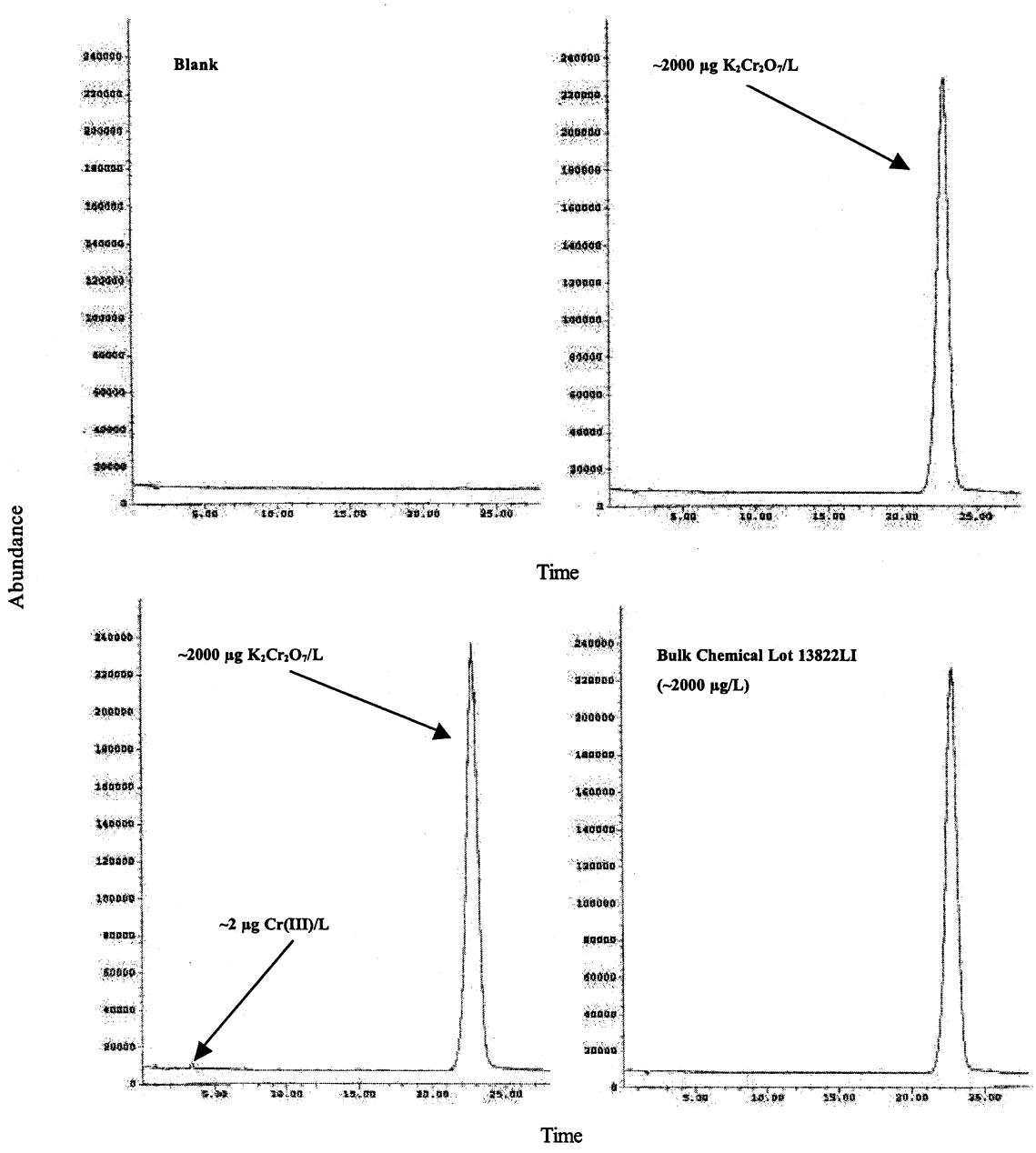

FIGURE F3
Liquid Chromatography-Inductively Coupled Plasma-Mass Spectrometry
Selected Ion-Current Profiles of Cr III and Cr VI in Sodium Dichromate Dihydrate

TABLE F1
Preparation and Storage of Dose Formulations in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate in F344/N Rats and B6C3F$_1$ Mice (Study 1)

Preparation
A premix was prepared in a beaker with tap water and then thoroughly mixed with additional tap water in a mixing tank. Dose formulations were prepared four times during the studies.

Chemical Lot Number
062001

Maximum Storage Time
42 days

Storage Conditions
Stored in NALGENE® containers at room temperature and protected from light.

Study Laboratory
Southern Research Institute (Birmingham, AL)

TABLE F2
Preparation and Storage of Dose Formulations in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate in Male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 Mice (Study 2)

Preparation
A premix was prepared in a beaker with tap water and then thoroughly mixed with additional tap water in a NALGENE® mixing tank. Dose formulations were prepared five times during the studies.

Chemical Lot Number
13822L1

Maximum Storage Time
39 days

Storage Conditions
Stored in NALGENE® containers and refrigerated at approximately 5° C.

Study Laboratory
Battelle Columbus Operations (Columbus, OH)

TABLE F3
Results of Analyses of Dose Formulations Administered to F344/N Rats and B6C3F$_1$ Mice in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate (Study 1)

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats				
November 2, 2001	November 5, 2001	62.5	62.1	–1
		125	129	+3
		250	257	+3
		500	508	+2
		1,000	1,024	+2
	December 11, 2001[b]	62.5	62.3	0
		125	125	0
		250	256	+3
		500	508	+2
		1,000	1,013	+1
	December 11, 2001[c]	62.5	61.8	–1
		125	130	+4
		250	257	+3
		500	522	+4
		1,000	1,009	+1
November 30, 2001	December 3, 2001	62.5	63.5	+2
		125	123	–1
		250	252	+1
		500	509	+2
		1,000	1,002	0
	January 8, 2002[b]	62.5	66.8	+7
		125	129	+3
		250	261	+4
		500	515	+3
		1,000	970	–3
	January 8, 2002[c]	62.5	67.3	+8
		125	132	+5
		250	263	+5
		500	510	+2
		1,000	1,015	+1

TABLE F3
Results of Analyses of Dose Formulations Administered to F344/N Rats and B6C3F$_1$ Mice in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate (Study 1)

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats (continued)				
January 25, 2002	January 28, 2002	62.5	64.1	+2
		125	128	+2
		250	251	+1
		500	490	–2
		1,000	959	–4
	February 15, 2002[b]	62.5	62.0	–1
		125	139	+11
		250	253	+1
		500	492	–2
		1,000	945	–5
	February 15, 2002[c]	62.5	64.1	+3
		125	128	+2
		250	253	+1
		500	488	–2
		1,000	956	–4
Mice				
November 2, 2001	November 5, 2001	62.5	62.1	–1
		125	129	+3
		250	257	+3
		500	508	+2
		1,000	1,024	+2
	December 11, 2001[b]	62.5	63.2	+1
		125	130	+4
		250	259	+4
		500	513	+3
		1,000	1,008	+1
	December 11, 2001[c]	62.5	61.8	–1
		125	130	+4
		250	257	+3
		500	522	+4
		1,000	1,009	+1

TABLE F3
Results of Analyses of Dose Formulations Administered to F344/N Rats and B6C3F$_1$ Mice in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate (Study 1)

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Mice (continued)				
November 30, 2001	December 3, 2001	62.5	63.5	+2
		125	123	–1
		250	252	+1
		500	509	+2
		1,000	1,002	0
	January 8, 2002[b]	62.5	67.1	+7
		125	131	+5
		250	259	+4
		500	520	+4
		1,000	1,014	+1
	January 8, 2002[c]	62.5	67.3	+8
		125	132	+5
		250	263	+5
		500	510	+2
		1,000	1,015	+1
January 25, 2002	January 28, 2002	62.5	64.1	+2
		125	128	+2
		250	251	+1
		500	490	–2
		1,000	959	–4
	February 15, 2002[b]	62.5	63.6	+2
		125	127	+2
		250	251	0
		500	489	–2
		1,000	954	–5
	February 15, 2002[c]	62.5	64.1	+3
		125	128	+2
		250	253	+1
		500	488	–2
		1,000	956	–4

[a] Results of duplicate analyses
[b] Animal room samples from drinking water bottles
[c] Unused samples from carboy storage containers

TABLE F4
Results of Analyses of Dose Formulations Administered to Male B6C3F$_1$, BALB/c, and *am3*-C57BL/6 Mice in the 3-Month Drinking Water Studies of Sodium Dichromate Dihydrate (Study 2)

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
August 5, 2002	August 7, 2002	62.5	66.65	+7
		62.5	64.83	+4
		125	131.3	+5
		125	131.9	+6
		250	257.4	+3
		250	259.9	+4
	September 18, 2002[b]	62.5	63.36	+1
		125	131.5	+5
		250	256.7	+3
	September 18, 2002[c]	62.5	63.56	+2
		125	131.1	+5
		250	256.2	+2
September 3, 2002	September 4, 2002	62.5	64.52	+3
		62.5	63.83	+2
		125	128.2	+3
		125	128.7	+3
		250	253.3	+1
		250	249.4	0
	October 11, 2002[b]	62.5	61.50	–1
		125	123.6	–1
		250	249.1	0
	October 11, 2002[c]	62.5	62.73	0
		125	125.1	0
		250	247.7	–1
November 4, 2002	November 4, 2002	62.5	64.35	+3
		62.5	66.55	+6
		125	127.8	+2
		125	126.5	+1
		250	258.3	+3
		250	257.0	+3
	November 26, 2002[b]	62.5	64.73	+4
		125	126.2	+1
		250	263.6	+5
	November 26, 2002[c]	62.5	64.02	+2
		125	126.1	+1
		250	259.2	+4

[a] Results of single analyses
[b] Animal room samples from drinking water bottles
[c] Unused samples from carboy storage containers

APPENDIX G
TOXICOKINETIC STUDIES

MATERIALS AND METHODS G-2
RESULTS AND DISCUSSION G-2
REFERENCE G-2
TABLE G1 Chromium Concentrations in the Blood, Bone, and Kidney of Male F344/N Rats, B6C3F$_1$ Mice, and Hartley Guinea Pigs Exposed to Chromium in Drinking Water for 21 Days G-3

TOXICOKINETIC STUDIES

MATERIALS AND METHODS

The NTP conducted a comparative absorption study of sodium dichromate dihydrate (Cr VI) administered in the drinking water to F344/N rats, B6C3F$_1$ mice, and Hartley guinea pigs. Concentrations of total chromium were determined in blood, kidney, and rat femur. Guinea pigs were chosen for study, in addition to rats and mice, because they more closely resemble humans in that they do not have a forestomach and they require vitamin C (a reducing agent) in their diet.

Twenty-eight male F344/N rats, B6C3F$_1$ mice, and Hartley guinea pigs between 6 and 10 weeks old were used in this study. Animals were approximately 6 to 7 weeks old at the time of receipt and were quarantined for approximately 6 to 7 days prior to placement on the study. Body weights at the start of the study were approximately 150 g for rats, 15 g for mice, and 40 g for guinea pigs.

Groups of four males of each species were provided *ad libitum* one of six concentrations of Cr VI in drinking water for 21 days, followed by 2 days on drinking water without added Cr VI. Controls were given water without added Cr VI. Body weights were collected on the first day of each study week (days 1, 8, 15, and 22) and at necropsy. Dose concentrations were 0, 2.87, 8.62, 28.7, 86.2, 287, and 862 mg CR VI/L (equivalent to 0, 1, 3, 10, 30, 100, and 300 mg chromium/L, respectively). Animals were sacrificed on day 24, and total chromium concentrations in blood, kidney, and femur (rats only) were determined.

RESULTS AND DISCUSSION

In all three species, chromium in blood and kidney increased with exposure concentration. Although differences were seen in the absolute amounts of chromium in kidney and blood, uptake as a function of exposure concentration did not appear to differ qualitatively in guinea pigs when compared to rats and mice. This suggests that the presence of a forestomach did not fundamentally alter the Cr VI handling in the gastrointestinal tract. Kidney chromium concentration was highest in rats, followed by mice and guinea pigs. For blood, guinea pigs had the highest concentrations followed by mice and rats. The concentrations of chromium in blood and kidney were in general agreement with values observed by Sutherland *et al*. (2000).

While differences were seen in the patterns of tissue chromium accumulation among the three species, the results for chromium concentrations in blood and kidney in rats and mice were in general agreement with expectations based on values reported in the literature. The chromium concentrations in the blood of guinea pigs suggested somewhat greater absorption than did the concentrations in the blood of the rats or mice.

REFERENCE

Sutherland, J.E., Zhitkovich, A., Kluz, T., and Costa, M. (2000). Rats retain chromium in tissues following chronic ingestion in drinking water containing hexavalent chromium. *Biol. Trace Elem. Res.* **74**, 41-53.

TABLE G1
Chromium Concentrations in the Blood, Bone, and Kidney of Male F344/N Rats, B6C3F$_1$ Mice, and Hartley Guinea Pigs Exposed to Chromium in Drinking Water for 21 Days[a]

	0 mg/L	1 mg/L	3 mg/L	10 mg/L	30 mg/L	100 mg/L	300 mg/L
n	4	4	4	4	4	4	4
Rat							
Blood	0.119 ± 0.021	0.146 ± 0.033[b]	0.124 ± 0.017[b]	0.148 ± 0.011	0.325 ± 0.026	0.510 ± 0.008[b]	0.653 ± 0.004[b]
Femur	1.22 ± 0.543	1.27 ± 0.233	0.744 ± 0.180[b]	1.79 ± 0.285	2.61 ± 0.296	3.38 ± 0.245[b]	4.42 ± 0.395[b]
Kidney	1.57 ± 2.38	0.488 ± 0.068	0.681 ± 0.063[b]	1.86 ± 0.231	4.59 ± 0.851	6.59 ± 0.465	9.44 ± 0.007[b]
Mouse							
Blood	0.367 ± 0.034	0.332 ± 0.028[b]	0.368 ± 0.029	0.402 ± 0.059	0.524 ± 0.050	0.784 ± 0.044[b]	1.10 ± 0.011[b]
Kidney	0.105 ± 0.220	0.243 ± 0.051[b]	0.350 ± 0.016	0.681 ± 0.088	1.39 ± 0.200	2.29 ± 0.042[b]	3.64 ± 0.042[b]
Guinea pig							
Blood	0.140 ± 0.021[b]	0.132 ± 0.035	0.152 ± 0.028	0.264 ± 0.153[b]	0.628 ± 0.143[c]	1.67 ± 0.046[b]	3.24 ± 0.049[b]
Kidney	0.132 ± 0.060	0.123 ± 0.011	0.129 ± 0.015	0.200 ± 0.011[b]	0.691 ± 0.344	1.50 ± 0.066[b]	1.70 ± 0.000[b]

[a] Data were calculated based on tissue mass: method blank corrected chromium µg /mL × final volume (10 mL)/mass digested (g) and are given in µg/g as mean ± standard error.
[b] Mean ± standard error adjusted to reflect duplicate analysis of one sample
[c] n=3