Final Report on the

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Southern Research Study Number: 13026.01.02

June 21, 2011

Final Report on the

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fisher Rats

To:

American Chemistry Council
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Arlington, VA 22209

and

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Katy, TX 77494

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P.O. Box 55305
Birmingham, AL 35255-5305

Executive Summary

Title:	90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fisher Rats
Study No.:	13026.01.02
Sponsor:	American Chemistry Council (Arlington, VA)
Sponsor's Representatives:	ToxStrategies, Inc. (Katy, TX) Mark R. Harris, Ph.D.; Laurie C. Haws. Ph.D., D.A.B.T.
Contractor:	Southern Research Institute (Birmingham, AL)
Study Director:	Charles D. Hébert, Ph.D., D.A.B.T.

Sodium dichromate dihydrate (SDD) is a form of hexavalent chromium [Cr(VI)] that is formed as a by-product of a variety of industrial processes and that is found as a contaminant in drinking water. In recent studies conducted by the National Toxicology Program (NTP)[1] administration of SDD in drinking water for 2 years was found to be associated with an increase in tumors of the oral epithelium in rats and of the intestinal epithelium in mice. The objective of this study was to evaluate the toxicity and potential mechanisms of action of SDD administered in drinking water to rats for 90 days. Female Fischer rats approximately 6-7 weeks of age on the first day of dosing received drinking water containing SDD at concentrations of 0, 0.3, 4, 60, 170, or 520 mg/L (equivalent to 0, 0.1, 1.4, 20.9, 59.3, and 181.4 mg Cr/L, respectively). Formulations were available ad libitum from Day 1 through the day of necropsy. These concentrations were similar to those used in the NTP studies with the exception of the 0.3 and 4 mg/L dose levels. The latter two concentrations were included in the current study to evaluate the mode of action at more relevant environmental exposure levels. One cohort of 25 rats/group was removed from study after 7 days of dosing (i.e., on Day 8), and was used for collection of samples for evaluation of histopathology, gene expression, reduced-to-oxidized glutathione ratio (GSH/GSSG ratio), or DNA-Cr adducts. The remaining rats were removed on Day 91, and samples were collected for evaluation of histopathology, iron status, gene expression, gene mutation, total chromium and iron content, and a variety of biochemical markers of oxidative stress and DNA-damaging potential including GSH/GSSG ratio, DNA-Cr adducts, 8–hydroxydeoxyguanosine (8-OHdG), 8-iso-prostaglandin F2α (8-isoprostane), and a panel of 23 cytokines/chemokines. Samples for analysis of GSH/GSSG ratio, DNA-Cr adducts, gene expression, gene mutation, and total chromium and iron content were shipped to Sponsor-designated laboratories for analysis, and the results of those evaluations are not presented in this report.

Administration of SDD had no effect on survival or food consumption of rats at any dose level, and there were no clinical signs that were considered to be related to SDD administration. Administration of SDD was associated with minimal deficits in body weight gain that were considered to be of no toxicological or biological significance, and by generally lower water consumption for rats in the 60, 170, and 520 mg/L groups.

No test article-related macroscopic lesions were observed at necropsy. On Day 8, SDD-related microscopic lesions in the duodenum included histiocytic cellular infiltration (170 and 520 mg/L), apoptosis (60, 170, and 520 mg/L), villous atrophy (170 and 520 mg/L), and crypt hyperplasia (60, 170, and 520 mg/L). In the jejunum, test article-related microscopic lesions included apoptosis (170 and 520 mg/L), villous atrophy (170 and 520 mg/L), and crypt hyperplasia (170 and 520 mg/L). On Day 91, SDD-related microscopic lesions in the duodenum included histiocytic cellular infiltration (60, 170 and 520 mg/L), apoptosis (60, 170 and 520 mg/L), villous atrophy (170 mg/L), and crypt hyperplasia (170 and 520 mg/L). In the jejunum, test article-related microscopic lesions included histiocytic cellular infiltration (60, 170 and 520 mg/L), apoptosis (170 mg/L), and crypt hyperplasia (170 and 520 mg/L). No microscopic lesions were observed in the oral mucosa on Day 8 or Day 91.

At levels ≥60 mg/L, SDD appeared to be related to decreases in mean serum iron levels (up to 20% lower than controls) on Day 91. Results from evaluation of Prussian blue-stained bone marrow smears from Day 91 also suggested decreased iron content/storage at the 170 and 520 mg/L dose levels. Taken together, the findings suggest that SDD may have been associated with iron deficiency in the rats. However, analyses for serum levels of ferritin and transferrin indicated that there were no SDD-related differences between the groups in terms of circulating levels of either of these proteins.

In the oral cavity or duodenum, there were no differences for 8-isoprostane levels between the vehicle control group and SDD-treated groups that were considered to be due to SDD administration. Similarly, there were no apparent differences between the vehicle control group and SDD-treated groups for 8-OHdG in oral cavity or duodenum. A statistically significant increase in levels of the pro-inflammatory cytokine IL-1α in the duodenum was observed for groups treated with 170 or 520 mg/L SDD, and statistically significant decreases in IL-12p70 and leptin in the serum were observed for groups treated with 60 and 520 mg/L SDD, respectively. These changes were also considered to be potentially related to SDD administration. No other remarkable changes occurred in the serum, oral cavity, or duodenum of any of the dose groups with respect to the 23 cytokines/chemokines analyzed.

Due to the microscopic changes observed in the duodenum and jejunum of animals in the 60 mg/L group on Day 91, as well as observed effects on water consumption and serum iron in the same dose group, the no observed effect level (NOEL) was determined to be 4 mg/L SDD under the conditions of this study.

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90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fisher Rats

Charles D. Hébert 6-21-11

Charles D. Hébert, Ph.D., D.A.B.T. Date
Study Director

GOOD LABORATORY PRACTICES DISCLAIMER

The study described in this final report was not conducted in strict compliance with the U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations (21 CFR Part 58), and neither the report nor the raw data were reviewed by the Southern Research Institute Quality Assurance Unit. However, the study was conducted according to the protocol and the applicable standard operating procedures, and all study procedures, data reporting, and recording were performed in a manner consistent with the standard of GLPs. The final report accurately reflects the raw data obtained during the performance of the study.

Charles D. Hébert, Ph.D., D.A.B.T. — Date
Study Director

Study Schedule and Personnel

Study Dates:

Study Initiation:	July 21, 2010
Treatment Initiation:	August 2-7, 2010; August 10, 2010; August 24-27, 2010
Study Termination:	August 9, 2010; September 2-3, 2010; November 1-5, 2010; November 8, 2010; November 22-23, 2010
Study Completion:	June 21, 2011

Study Personnel:

Charles D. Hébert, Ph.D., D.A.B.T.	Study Director
Kellye K. Daniels, Ph.D., D.A.B.T.	Director, Toxicology
Brian E. LaGory, B.S.	Supervisor, Toxicology Animal Laboratory
Dora Marrisette, B.S.	Team Lead, Toxicology Animal Laboratory
Christy L. Price, B.S.	Supervisor, Preclinical Research Support (September 27, 2010 – Present)
Courtney R. Goslowsky, B.S., ALAT	Preclinical Research Coordinator
Gregory S. Gorman, Ph.D.	Director, Bioanalytical Sciences (July 21, 2010 – July 30, 2010)
Lori U. Coward, B.S.	Supervisor, Bioanalytical Sciences (July 21, 2010 – July 30, 2010)
Sanford Mendonca, Ph.D.	Manager, Bioanalytical Sciences (August 2, 2010 – Present)
Russell Carter, B.S., ASPC	Supervisor, Clinical Pathology (July 21, 2010 – September 17, 2010)
Brenda Yamamoto, D.V.M., Ph.D., D.A.C.V.P.	Clinical Pathologist
Jill F. Mann, D.V.M., D.A.C.V.P.	Senior Research Pathologist
Richard D. May, Ph.D.	Manager, Cell Biology and Immunology
James S. Toomey, D.V.M.	Attending Veterinarian
D. Wayne May, RLATG	Supervisor, Animal Care
Nicola Richardson-Harman, Ph.D.	Consultant Statistician

1.0 Introduction

Sodium dichromate dihydrate (SDD) is a form of hexavalent chromium [CR(VI)] that is produced as a result of various industrial processes and is found as a contaminant in drinking water. In recent studies conducted by the National Toxicology Program[(1)] administration of SDD in drinking water for 2 years was found to be associated with an increase in tumors of the oral epithelium in rats and of the small intestinal epithelium in mice. The objective of this study was to evaluate the toxicity and potential mechanisms of action of sodium dichromate dihydrate (SDD) administered in drinking water to rats for 90 days. A copy of the operational protocol and amendments are presented in Appendix A.

2.0 Materials and Methods

The Provantis application (Version 7; Instem Life Sciences Systems, Ltd.; Staffordshire, United Kingdom) was used for the direct on-line capture of most in-life and pathology data. In addition, Provantis interfaced with the Cobas c501 Clinical Chemisty Analyzer (Version 04-02; Roche Diagnostics; Indianapolis, IN) for the capture of serum iron data. Environmental monitoring of animal rooms (i.e., temperature/humididty and light/dark cycles) was performed using the Edstrom Watchdog system (Version 5.13; Edstrom Industries, Inc.; Waterford, WI). The remainder of the data was collected manually.

2.1 Test System

The 450 female Fischer rats designated for use in this study were selected from 595 females received in two separate shipments from Charles River Laboratories International, Inc. (Stone Ridge, NY). The rats were approximately 4 weeks of age when they arrived at Southern Research Institute (Southern Research) on July 20, 2010 and August 10, 2010. The animal identification number for each rat consisted of a number designating the SDD treatment group, a letter designating the sex, and a unique number (e.g., 1F12). The rats were uniquely identified by tail tattoo using the unique numerical portion, but not the SDD treatment group or sex letter portion, of the identification. Prior to the start of the study, animals were observed for general health and acceptability for use in this study. There were no findings indicative of poor health and the Fischer rats were deemed suitable for use on this study by the Veterinarian. The Fischer rat is an accepted species and strain that is commonly used in toxicological evaluations of

compounds to which humans may be exposed. On Day 1 of the study (8/2/10-8/7/10; 8/10/10; 8/24/10-8/27/10), the rats were approximately 6-7 weeks old and weighed between 83.1 and 126.4 grams.

Irradiated NTP-2000 Wafers (Zeigler Bros.; Gardners, PA) were provided ad libitum to the rats during the pre-study and study periods. Analysis of the feed was conducted by the manufacturer. The results of the feed analysis are located in the facility records at Southern Research. Water (Birmingham public water supply), either undosed for control animals or containing SDD for treated animals, was supplied in glass water bottles protected from light. Teflon®-lined plastic screw caps with stainless steel, double-balled sipper tubes were used. Water bottles were changed twice weekly on a 3-day/4-day schedule, or more frequently as needed. Samples of water from the animal facility were periodically analyzed, and the analyses were reviewed by Southern Research's Attending Veterinarian or designee. No known contaminants were present in the food or water that would have been expected to interfere with or affect the outcome of the study.

The animals were group housed (5/cage) in solid bottom cages on stainless steel racks in a room maintained at a temperature of 60.7–84.1 °F and a relative humidity of 28.4%–100.0%. Excursions from the protocol-specified temperature (69-75 °F) and humidity (35%-65%) ranges were generally minor and were deemed to have no impact on the health of the animals (see Comments on Study Data). Fluorescent lighting provided illumination approximately 12 hours per day. Irradiated hardwood bedding chips (Sani Chips®; P.J. Murphy Forest Products, Corp.; Montville, NJ) was used as bedding material. No known contaminants were present in the bedding that would have been expected to interfere with or affect the outcome of the study. Cage size and animal care conformed to the guidelines of the *Guide for the Care and Use of Laboratory Animals*,[(2)] the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198), and to the applicable Standard Operating Procedures (SOPs) of Southern Research.

2.2 Test Article and Vehicle

2.2.1 Test Article: Southern Research received 27 bottles (10 grams/bottle) of Sodium Dichromate Dihydrate (SDD) (Lot No. 05914AS; Southern Research Lot No. E36/L-4; expiration date unknown) on February 19, 2010. The SDD was supplied by Sigma Aldrich, Inc. (Milwaukee, WI). The test article was received at room temperature and was stored at room temperature and protected from light. A copy of the Certificate of Analysis is presented in Appendix B.

2.2.2 Vehicle: The tap water used in this study was supplied from the Birmingham Water Works. Water was not stored prior to use in preparation of dose formulations.

2.2.3 Dose Formulation Preparation: The SDD dose formulations were prepared at a concentration of 0.3, 4, 60, 170, and 520 mg/L in tap water. A premix was prepared for each concentration by mixing the required amount of SDD in tap water until dissolution. The premix was then transferred to a mixing container which had been filled with a portion of the required amount of tap water (about two-thirds full). After the premix container was rinsed with tap water five times, and the rinseate was transferred each time to the mixing container, the contents of the mixing container were then stirred (~2 minutes). The mixing container was then brought to final volume and the formulations stirred an additional 5 minutes. Dose formulations of SDD were prepared and vehicle control formulations (tap water) were collected once during Week -2 and at 2-week (i.e., 10- to 17-day) intervals thereafter throughout the study.

2.2.4 Dose Formulation Concentration Analysis: Samples of each batch of vehicle or SDD dose formulations from the first, third, fifth, and last mixes were collected and shipped to Brooks Rand Labs (Seattle, WA) for concentration analysis. The results of these analyses were provided to Southern Research by the Sponsor. Because dose formulations of SDD in tap water are solutions, it was not necessary to demonstrate homogeneity of the formulations used in this study. Information on the designated laboratory was included in the study records.

2.2.5 Formulation Storage, Stability, and Handling: When not in use, dose formulations of SDD and vehicle formulations were stored in sealed Nalgene containers at room temperature protected from light. SDD has been shown to be stable for 42 days in dosed water formulations at a concentration of 41.8 mg/L when stored under these conditions.[(1)] Reserve samples of each formulation were retained and stored at approximately -70 °C and protected from light.

SDD formulations in tap water have been shown to be stable under simulated animal room conditions (i.e., ambient temperature in glass bottles) for at least 7 days.[(1)]

Disposition: Residual formulations remaining after dose administration was complete were disposed of as hazardous waste.

2.3 Experimental Design

2.3.1 Randomization and Group Assignment: Animals were assigned to their respective treatment groups using a computerized randomization procedure designed to yield comparable group mean body weights. Because of the number of rats in the study, the animals were received in two separate shipments (cohorts), and the rats in the different cohorts were randomized separately. The body weights required for randomization were determined during the week prior to randomization. After randomization, animals were assigned to treatment groups as indicated below. The correlation between animal identification numbers, cage numbers, dose levels, and analysis groups is shown in Table 1.

Group	Treatment	Conc. (mg/L)	Number of Animals							
			Toxicology and Histopathology		Biochemical Evaluations		Gene Expression Analysis		Mutation Analysis	Chromium/ Iron Analysis
			Day 8	Day 91	Day 8	Day 91	Day 8	Day 91	Day 91	Day 91
1	Water	0	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
2	SDD	0.3	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
3	SDD	4	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
4	SDD	60	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
5	SDD	170	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
6	SDD	520	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F

Color codes and number designations were assigned to SDD treatment groups as follows:

Group	Treatment	Conc. (mg/L)	Color Code
1	Water	0	Black
2	SDD	0.3	Grey
3	SDD	4	Yellow
4	SDD	60	Purple
5	SDD	170	Blue
6	SDD	520	Red

2.3.2 Dose Procedure: In order to accommodate necropsy and sample collections on large numbers of rats the study was stagger-started, with Days 1 distributed as shown below.

Days 1

Event	Sequence	Dates
Day 1 of Dosing	Toxicology/Histology Groups (Day 8 Necropsy, 5/group)	8/27/10
	Toxicology/Histology Groups (Day 91 Necropsy, 5/group)	8/3/10
	Toxicology/Histology Groups (Day 91 Necropsy, 5/group)	8/4/10
	Biochemical Evaluation Groups (Day 8, 10/group)	8/26/10
	Biochemical Evaluation Groups (Day 91, 10/group)	8/5/10
	Biochemical Evaluation Groups (Day 91, 5/group)	8/6/10
	Gene Expression Groups (Day 8, 10/group)	8/2/10
	Gene Expression Groups (Day 91, 10/group)	8/7/10
	Mutation Analysis Groups (Day 91, 5/group)	8/24/10
	Mutation Analysis Groups (Day 91, 5/group)	8/25/10
	Total Chromium and Iron Analyses (Day 91, 5/group)	8/10/10

Rats in this study received the test article in their drinking water. The test article was available ad libitum to study animals 7 days per week (including holidays) for 7 or 90 days, as shown in the table above.

2.3.3 Clinical Observations: All animals were observed at least twice daily during the pre-study and study periods for signs of mortality and moribundity. Each animal was removed from its cage and examined for clinical signs of toxicity on Day 1 and weekly thereafter.

2.3.4 Body Weights: Each animal was weighed during Week -1 for randomization, on Day 1, weekly thereafter, and prior to scheduled euthanasia.

2.3.5 Food and Water Consumption: Quantitative food and water consumption were measured by cage weekly for each cage of animals throughout the study. Values were reported as an average consumption [(grams/animal/day) or (mL/animal/day), respectively] on a weekly basis. For comparison with values from NTP studies, water consumption values in the current study were also calculated as an average over the entire 13 weeks of dosing, corrected for the actual number of values at each time point. This transformation is documented in the study files and the transformed water consumption values are shown only in the Discussion of this report for comparison with values from NTP studies.

2.3.6 Mutation Analysis: Samples for mutation analysis were collected on Day 91; in addition, five rats/group from the animals used for mutation analysis will be used for collection of samples for evaluation of iron status as described below. Ten rats/group were euthanized using CO_2, and samples of oral epithelium and duodenal epithelium were collected and snap frozen. These samples were stored frozen at approximately -80 °C or lower until they were shipped for analysis to a Sponsor-designated laboratory at the following address:

Dr. Travis O'Brien
Department of Pharmacology and Physiology
George Washington Cancer Institute
Washington, DC 20037

2.3.7 Gene Expression Analysis: Samples for gene expression analysis were collected on Days 8 and 91. On each of these days, 10 rats/group were euthanized using CO_2, and samples of oral epithelium, duodenal epithelium, and jejunal epithelium were collected and snap frozen. These samples were stored frozen at approximately -80 °C or lower until they were shipped for analysis to a Sponsor-designated laboratory (laboratory of Dr. Tim Zacharewski) at the following address:

Anna K. Kopec
Michigan State University
East Lansing, MI 48824

Following collection of these samples, carcasses and remaining tissues from rats designated for gene expression analysis were discarded without further evaluation.

2.3.8 Biochemical Analysis:

Sample Collection: Samples for biochemical analyses were collected from 10 rats/group on Day 8 and from 15 rats/group on Day 91. On Days 8 and 91, 10 rats/group were designated as Subgroup A; on Day 91, the remaining 5 rats/group were designated as Subgroup B.

On Day 8 and on Day 91, 5 rats/group in Subgroup A were used for collection of samples for GSH/GSSG analysis and 5 rats/group in Subgroup A were used for collection of samples for DNA-Cr adduct analysis.

Collection of Blood for GSH/GSSG Analysis (Subgroup A): For collection of blood samples for GSH/GSSG analysis, each rat was anesthetized with ketamine/xylazine (87 mg ketamine/kg; 13.4 mg xylazine/kg) injected intraperitoneally and blood samples were collected from the retro-orbital plexus into tubes containing heparin as anticoagulant (Subgroup A). Samples were gently mixed by inversion and placed on ice. Within 15

minutes of collection, samples were centrifuged for approximately 5 minutes under refrigerated conditions for separation of plasma. Plasma was collected, and mixed in a 1:1 ratio with 2X Redox Quenching Buffer (RQB), to yield final concentrations of 20 mM HCl, 5 mM diethylenetriamine pentaacetic acid, and 1 mM 1,10-phenanthroline. The 2X RQB also contained 5% ultrapure grade trichloroacetic acid. Samples were snap frozen until they were shipped for analysis.

Collection of Tissues for GSH/GSSG and DNA-Cr Adduct Analysis (Subgroup A): Immediately following blood collection, each rat was euthanized using CO_2. Samples of oral epithelium, duodenal epithelium, and jejunal epithelium were collected from animals in Subgroup A. Tissues for GSH/GSSG analysis were immediately placed into tubes containing 0.5 mL 2X RQB on ice. The tissues were allowed to sit in RQB on ice for approximately 10-15 minutes to allow penetration of the buffer into the tissues, then the tubes were snap frozen in liquid nitrogen. For animal 6F400 samples of duodenal epithelium and jejuna epithelium were allowed to sit in buffer on ice for nine minutes and seven minutes, respectively, prior to freezing. Tissues for DNA-Cr adduct analysis were placed into tubes and snap frozen without buffer.

Collection of Blood for Cytokine Analysis (Subgroup B): For collection of blood samples for cytokine analysis, each rat was anesthetized with CO_2/O_2, and blood samples were collected from the retro-orbital plexus into serum separator tubes containing no anticoagulant (Subgroup B). The contents of the Subgroup B tubes were centrifuged to separate serum.

Collection of Tissues for 8-OHdG, Cytokine, and 8-Isoprostane Analysis (Subgroup B): A sample of oral mucosa and underlying muscle and an intact segment from the cranial end of the duodenum was collected from each animal in Subgroup B. Following collection of these tissues, rats designated for biochemical analysis will be discarded without further evaluation.

Plasma and tissue samples from animals in Subgroup A were stored frozen (at or below -70 ºC) until they were shipped to Sponsor-designated laboratories for analysis. Serum samples from animals in Subgroup B were divided into two aliquots, and the oral cavity and duodenum samples were weighed and split into two pieces longitudinally. Serum and tissue samples were snap-frozen upon collection, and were stored frozen (at or below -80 ºC) until they were used for analysis. Weights of the tissue pieces are shown in Table 2.

Biochemical Analysis, GSH/GSSG Ratio: One plasma sample, one sample of oral epithelium, one sample of duodenal epithelium, and one sample of jejunal epithelium from each of 5 animals/group (Day 8 and Day 91 collections) in Subgroup A were shipped to a Sponsor-designated laboratory at the address shown below for analysis of GSH and GSSG, and subsequent calculation of GSH/GSSG ratios.

Dr. Howard G. Shertzer
Division of Environmental Genetics & Molecular Toxicology
University of Cincinnati Medical Center
Cincinnati, OH 45267-0056

Biochemical Analysis, DNA-Cr Adducts: One sample of oral epithelium, one sample of duodenal epithelium, and one sample of jejunal epithelium from each of 5 animals/group (Day 8 and Day 91 collections) in Subgroup A were shipped for analysis to a Sponsor-designated laboratory at the following address: :

Dr. Travis O'Brien
Department of Pharmacology and Physiology
George Washington Cancer Institute
Washington, DC 20037

Biochemical Analysis, 8-OHdG: One sample of oral cavity and one sample of duodenum from each animal in Subgroup B was analyzed for 8-OHdG as described in Appendix G.

Biochemical Analysis, Cytokines: One serum sample, one sample of oral cavity, and one sample of duodenum from each animal in Subgroup B was analyzed for IL-1α, IL-

1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-15, IL-17, IL-18, TNF-α, KC/GRO, MCP-1, G-CSF, GM-CSF, IP-10, MIP-1α, RANTES, eotaxin, leptin, VEGF, and IFN-γ as described in Appendix G.

Biochemical Analysis, 8-Isoprostane: One sample of oral cavity and one sample of duodenum from each animal in Subgroup B was analyzed for 8-isoprostane as described in Appendix G.

2.3.9 Total Chromium and Iron Analysis: Samples for evaluation of total chromium and iron content were collected on Day 91. Five rats/group were anesthetized with CO_2/O_2, and blood was collected from the retro-orbital plexus into tubes containing lithium heparin as anticoagulant. Samples were gently mixed by inversion and placed on ice until they were centrifuged to separate plasma. The erythrocyte layer and the plasma layer was collected separately, snap-frozen, and stored at approximately -20 °C.

Following blood collection, rats were euthanized using CO_2, and organs/tissues were collected intact, weighed, snap-frozen, and stored at approximately -80 °C. Prior to freezing, the length of the intestinal segments (duodenum, ileum, and jejunum) was recorded (see Comments on Study Data for exception). The following organs/tissues were collected:

Bone (femur)
Glandular stomach (flushed of contents)
Kidney
Liver
Oral mucosa
Small intestine, duodenum (flushed of contents)
Small intestine, jejunum (flushed of contents)
Small intestine, ileum (flushed of contents)
Spleen

Plasma, red blood cells, and tissue samples were stored frozen at approximately -80 °C until they were shipped to a Sponsor-designated laboratory for analysis at the address

shown below. Bone (femur) and kidney samples were retained at Southern Research pending instructions on disposition from the Sponsor.

Andrea Pratt
Brooks Rand Labs
3958 6th Avenue NW
Seattle, WA 98107

Following collection of these tissues, rats designated for these groups were discarded without further evaluation.

2.3.10 Evaluation of Iron Status: Prior to euthanasia, half of the rats designated for macroscopic and microscopic pathology evaluation (i.e., those in the Toxicology and Histopathology groups), and half of the rats designated for mutation analysis were also used for collection of blood samples for evaluation of iron status.

Sample Collection: On Day 91, five rats per group were anesthetized using CO_2/O_2, and blood samples (~1.0 mL) was collected from the retro-orbital plexus into serum separation tubes (see Comments on Study Data). The contents of the tubes was centrifuged to separate serum. Serum samples were aliquotted into four aliquots, one of which was used for measurement of serum iron on the day of collection. The remaining three aliquots were snap frozen and stored at approximately -20 °C until used for ELISA analysis.

After collection of serum samples, rats were euthanized for gross and microscopic pathology as described below, or for mutation analysis as described previously. One bone marrow smear was prepared from each rat in the Toxicology and Histopathology groups, and three bone marrow smears were prepared from each rat in the mutation analysis groups. In addition, one femur from each rat in the mutation analysis groups were collected, fixed in formalin, and retained for possible future use.

Measurement of Serum Iron: One serum aliquot was used for measurement of serum iron levels using the Cobas c501 Clinical Chemistry Analyzer (Version 04-02; Roche Diagnostics; Indianapolis, IN).

Evaluation of Bone Marrow Smears and Formalin-Fixed Bone: Bone marrow smears were stained using a Prussian Blue stain (Iron Stain Kit; ScyTek Laboratories; Logan, UT) that allowed visualization of iron, and were evaluated by a board-certified clinical pathologist to estimate iron content.

ELISA Analysis of Serum Samples: Two frozen serum samples collected from the rats used for mutation analysis were analyzed for serum ferritin and serum transferrin using commercial ELISA kits. The third was retained for possible future later analysis of serum hepcidin. The frozen serum samples collected from the rats used for gross and microscopic pathology were retained for possible future use.

2.3.11 Macroscopic and Microscopic Pathology: Rats designated for macroscopic and microscopic pathology evaluation (i.e., those in the Toxicology and Histopathology groups) were euthanized by CO_2 asphyxiation on Day 8 (5 rats/group) and Day 91 (10 rats/group).

Rats in the groups designated for pathologic examination that were euthanized at scheduled necropsy and were subjected to a complete gross necropsy examination. The postmortem examination of each rat included, but was not limited to, examination of the external surfaces of the body, all orifices of the body, and the cranial, thoracic, abdominal, and pelvic cavities and their contents.

The oral cavity, duodenum, jejunum, and any gross lesions were collected from each rat and saved in 10% neutral buffered formalin for histopathologic evaluation. The animal identification was collected, fixed in 10% neutral buffered formalin, and retained with its tissues collected during necropsy.

In addition, for animals in histopathology groups 1, 2, 4, and 6 on Days 8 and 91, the esophagus, stomach (forestomach and glandular), liver, and mesenteric lymph nodes were collected. Each tissue was divided into two samples and saved for possible future evaluation. One piece of each tissue from each animal was fixed in 10% neutral buffered formalin, and the other was snap frozen and stored at -80 °C or lower.

Histology: The oral cavity, duodenum, jejunum, and any gross lesions from each rat in the Toxicology and Histopathology groups were processed to slides. The fixed tissues were trimmed, processed, and microtomed (approximately 5-µm sections), and the tissue sections were mounted on glass slides; ten slides of each oral cavity, duodenum, and jejunum were prepared, and one slide for gross lesions. One of the ten slides from each oral cavity, duodenum, and jejunum of each animal, or one slide for each gross lesion, was stained with hematoxylin and eosin, and coverslipped. The remaining nine samples of oral cavity, duodenum, and jejunum were shipped to a Sponsor designated laboratory at the following address:

Dr. Travis O'Brien
Department of Pharmacology and Physiology
George Washington Cancer Institute
Washington, DC 20037

Microscopic Observations: All slides were submitted to a veterinary pathologist for evaluation and diagnosis. For tissues from animals in the Toxicology and Histopathology groups, findings were diagnosed and categorized using standardized nomenclature with lesions ranked for severity for comparison among groups.

2.3.12 Statistical Analysis: Group means and standard deviations were calculated when appropriate for body weights, food and water consumption data, 8-isoprostane and 8-OHdG data, serum iron data, plasma ferritin and transferrin data, and cytokine/chemokine data. Evaluation of data for the differences between groups was performed for data sets in which there were at least three values in the control and at least one SDD concentration group.

The Kolmogorov-Smirnov test (alpha = 0.001) was used to test whether the food consumption, water consumption, body weight, 8-isoprostane, 8-OHdG, serum iron, plasma ferritin, and plasma transferrin data were normally distributed. Data for 8-isoprostane in the oral cavity were found to have unequal variances; therefore, these data were $\log_{10}$ transformed prior to stastical analysis. All data for water consumption, food consumption, body weights, 8-OHdG (oral cavity and duodenum), 8-isoprostane (oral cavity [transformed] and duodenum), serum iron, plasma ferritin, and plasma transferrin met criteria for normality, and for these data a one-way Analysis of Variance (alpha = 0.05) was performed at each timepoint followed by a post-hoc Dunnett test (alpha = 0.05) to compare Groups 2-6 to Group 1. SAS™ Version 9.2 and an alpha = 0.05 were used for all inter-group comparisons.

Statistical analysis for cytokine/chemokine data was performed using the Provantis automated data collection system (Instem; Staffordshire, UK) at Southern Research. Statistical analysis for food consumption, water consumption, body weight, 8-isoprostane, 8-OHdG, serum iron, plasma ferritin, and plasma transferrin data data was performed by a consultant statistician as shown below, and the Statistics Contributing Scientist Report containing the results of these analyses in presented in Appendix J.

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Calculation of summary food and water consumption values (i.e., means and standard deviations) followed the guidelines below:

1. For any cage that had a comment in the data indicating that the feeder or the water bottle had been spilled, that a feeder had been contaminated, or that animals were unable to access food or water, the calculated food or water consumption value for that cage for that week was not included in the calculation of means and standard deviations or in statistical analysis.
2. For any group that had fewer than three food or water consumption values at any given time point, a mean but not a standard deviation was calculated.

3.0 Results

3.1 Dose Formulation Concentration Analysis

The results of the dose formulation concentration analyses are presented in Table 3. The concentrations of SDD in all dose formulations used in this study were within ± 10% of the target concentrations.

3.2 Mortality

Summary and individual mortality data are included in Table 4 and Appendix C, respectively. Administration of SDD had no effect on survival of rats.

3.3 Clinical Observations

Summary and individual clinical observations data are presented in Table 4 and Appendix C, respectively. There were no clinical signs of toxicity in this study that were considered to be related to administration of SDD.

Clinical signs that were observed included alopecia, eye discharge, piloerection, hyperexcitability, and discoloration (vulva, one animal). These signs occurred in a sporadic and non-dose-related manner, and were considered to be incidental and not related to SDD administration.

3.4 Body Weights

Summary and individual body weight data are presented in Table 5 and Appendix D, respectively. Administration of SDD was associated with minimal deficits in body weight gain for rats in the 520 mg/L group.

Mean body weights for rats in the 520 mg/L group were generally lower than those of rats in the 0 mg/L group, starting on Day 22 and continuing to the end of the study. However, these differences were minimal, and rose to the level of statistical significance only sporadically during the study (Days 36, 43, 85, and 91). While these deficits in body weight gain were

considered to be potentially related to SDD administration, the maximum difference between mean body weights of rats in the 520 mg/L group and those in the vehicle control group was <4%. Therefore, the observed deficits in body weight gain were considered to be of no toxicological or biological significance.

A single statistically significant reduction in body weight observed for rats in the 170 mg/L dose group on Day 78 was considered to be incidental, and not related to SDD administration.

3.5 Food and Water Consumption

Summary and individual food consumption data are presented in Table 6 and Appendix E, respectively. Summary and individual water consumption data are presented in Table 7 and Appendix F, respectively. Administration of SDD had no effect on food consumption of rats at any dose level. Reductions in water consumption were observed for rats in the 60, 170, and 520 mg/L groups; these reductions were generally dose-related, and were considered to be related to SDD administration.

Food consumption values for rats in the 520 mg/L group were statistically elevated compared to controls for the Day 43-50 and Day 57-64 periods. However, because these elevations were minimal (≤ 10% different from control) and occurred at only two time points in the study, they were not considered to be related to SDD administration.

Water consumption was statistically reduced for rats in the 60 mg/L group for Days 22-50, 71-78, and 85-91, and for rats in the 170 and 520 mg/L groups at all time points in the study, when compared to the control group. Overall water consumption for the entire study for these three SDD treatment groups was approximately 92%, 79% and 73%, respectively, of that for rats in the control group. Mean water consumption values for rats in the 170 and 520 mg/L groups were on occasion as much as 26% and 31% lower, respectively, than those of rats in the vehicle control group. Because of the dose-related pattern of the changes, the reductions in water consumption for rats in the 60, 170, and 520 mg/L groups were considered to be related to SDD administration.

3.6 Biochemical Analysis

Data from the evaluation of serum, oral cavity, and duodenum for selected biochemical parameters are presented in the Immunology Contributing Scientist Report in Appendix G. 8-isoprostane, 8-OHdG, and cytokine levels were measured in oral cavity and duodenum; in addition, cytokine levels were measured in serum samples collected at necropsy.

8-Isoprostane: Individual results of 8-isoprostane assays conducted on oral cavity and duodenum samples are presented in Table G3, and summary results are presented in Table G4. The results indicated that there were no SDD-related differences in 8-isoprostane levels in the oral cavity or duodenum between treated groups and the vehicle control group.

8-OHdG: Individual results of 8-OHdG assays conducted on oral cavity and duodenum samples are presented in Table G3, and summary results are presented in Table G4. Data are shown normalized to the amount of DNA in each sample. There were no apparent differences in 8-OHdG levels in the oral cavity or duodenum between treated and control groups.

Cytokines/Chemokines: The results of assays for 23 cytokines/chemokines that were conducted on serum, oral cavity, and duodenum samples are presented in Table G5 and Table G6 (individual and summary serum data, respectively), Table G7 and Table G8 (individual and summary oral cavity data, respectively), and Table G9 and Table G10 (individual and summary duodenum data, respectively). Levels of many of the cytokines/chemokines were at low or background levels in all three sample types tested. Cytokines/chemokines that were found at notable levels in serum included GRO/KC (the rat equivalent of human IL-8), IL-2, IL-6, IL-12p70, leptin, MCP-1, and RANTES. The only statistically significant differences observed for cytokines/chemokines in serum were decreases in IL-12p70 for rats in the 60 mg/L group and leptin for rats in the 520 mg/L group. It is noteworthy that the levels of IL-12p70 were below the detectable limit (and thus not amenable to statistical analysis) for all animals in the two higher groups, suggesting that the decrease observed at 60 mg/L may have been part of a negative dose-related trend. For this reason, and because the decrease in leptin occurred only at the highest dose, both these changes were considered to be potentially related to SDD administration.

Cytokines/chemokines that were found at notable levels in oral cavity homogenates included IL-1α, IL-18, leptin, and VEGF. There were no statistically significant differences between control animals and animals administered SDD for cytokine/chemokine levels in the oral cavity. Cytokines/chemokines that were found at notable levels in duodenum homogenates included IL-1α, IL-1β, IL-18, leptin, RANTES, and VEGF. The only statistically significant difference for cytokine/chemokine levels in duodenum homogenates was an increase in IL-1α levels that was observed for rats in the 170 and 520 mg/L groups. However, the levels of IL-1α in duodenum homogenates also appeared to be elevated, although not statistically so, for animals in the 60 mg/L group. The changes in IL-1α levels were considered to be potentially due to SDD administration.

GSH/GSSG Ratio and DNA-Cr Adducts: The results of assays for GSH/GSSG ratio and for DNA-Cr adducts will be reported to the Sponsor directly by the laboratories performing those assays, and will not be included in this study report.

3.7 Macroscopic Pathology

The Contributing Scientist Report for pathology is presented in Appendix H with individual and summary macroscopic pathology data provided in Table H1 and Table H2, respectively. No macroscopic lesions associated with the oral administration of SDD were observed on Day 8 or on Day 91.

3.8 Microscopic Pathology

The Contributing Scientist Report for pathology is presented in Appendix H with individual and summary microscopic pathology data provided in Table H3 and Table H4, respectively. Test article-related microscopic lesions were crypt epithelial hyperplasia, histiocytic cellular infiltration of the villous lamina propria, villous atrophy, and apoptosis.

On Day 8, SDD-related microscopic lesions were observed in the small intestine (duodenum or jejunum). In the duodenum, test article-related microscopic lesions included minimal to mild histiocytic cellular infiltration (170 or 520 mg/L), minimal to mild apoptosis (60, 170, or 520 mg/L), minimal to mild villous atrophy (170 or 520 mg/L), and minimal to

moderate crypt hyperplasia (60, 170, or 520 mg/L). In the jejunum, test article-related microscopic lesions included minimal to mild apoptosis (170 or 520 mg/L), minimal villous atrophy (170 or 520 mg/L), and minimal to mild crypt hyperplasia (170 or 520 mg/L). In the jejunum, test article-related microscopic lesions included minimal apoptosis, minimal villous atrophy, and minimal to moderate crypt hyperplasia.

On Day 91, SDD-related microscopic lesions were observed in the small intestine (duodenum or jejunum). In the duodenum, test article-related microscopic lesions included minimal to marked histiocytic cellular infiltration (60, 170, or 520 mg/L), minimal to mild apoptosis (60, 170, or 520 mg/L), mild villous atrophy (170 mg/L), and minimal crypt hyperplasia (170 or 520 mg/L). In the jejunum, test article-related microscopic lesions included minimal to mild histiocytic cellular infiltration (60, 170, or 520 mg/L), minimal apoptosis (170 mg/L), and minimal crypt hyperplasia (170 or 520 mg/L). Lesions that were considered to be potentially, but not definitively, test article-related were observed in the jejunum of one animal in the 4 mg/L dose group, and included minimal apoptosis, moderate villous atrophy, and minimal crypt.

No test article-related microscopic lesions were observed in the oral mucosa of rats in any of the SDD treatment groups on Days 8 and 91. Because of the apoptosis and villous atrophy seen in the jejunum on Day 91, the no observable effect level was considered to be 0.3 mg/L SDD.

3.9 Iron Status

Measurement of Serum Iron: Data on serum iron levels are presented in the Clinical Pathology Contributing Scientist Report in Appendix I. Overall, mean serum iron levels were decreased relative to the control group mean in groups of rats receiving SDD doses of 60 mg/L or greater, although the decrease for rats in the 60 mg/L dose group did not rise to the level of statistical significance. Furthermore, changes in serum iron concentrations did not show a dose-dependent relationship. These results suggest that there may have been a test article-related decrease in serum iron levels with administration of 60 mg/L or greater SDD in the drinking water to rats.

Evaluation of Bone Marrow Smears: Data from the evaluation of bone marrow smears for iron are presented in the Clinical Pathology Contributing Scientist Report in Appendix I. As assessed by examination of Prussian blue-stained bone marrow smears, SDD administration had no discernible impact upon stored iron for rats in the 0.3, 4, and 60 mg/L groups. However, rats in the 170 mg/L SDD administration group showed slightly lower overall levels of detectable iron than controls (with grades of 2-3, 2-3, 2-3, 3, and 3 for the five rats in the 170 mg/L group compared to grades of 2-3, 3, 3, 3, and 3 for rats in the control group). Rats in the 520 mg/L group had consistently lower levels of detectable iron on the bone marrow smear, with all rats in this SDD treatment group having iron grades of 2.

ELISA Analysis of Serum Samples: Data on serum ferritin and transferrin levels are presented in the Immunology Contributing Scientist Report in Appendix G. Individual results are presented in Table G1 and summary results are presented in Table G2. These results and the statistical analyses indicated that there were no SDD-related differences between the groups in terms of circulating ferritin or transferrin levels.

3.10 Gene Expression Analysis

The results of assays for gene expression will be reported to the Sponsor directly by the laboratory performing those assays, and will not be included in this study report.

3.11 Total Chromium and Iron Analysis

The results of assays for total chromium and iron content in RBC, plasma, and tissues will be reported to the Sponsor directly by the laboratory performing those assays, and will not be included in this study report. Individual and summary values for organ weights and lengths of intestinal segments collected for total chromium and iron analysis are presented in Table 8.

3.12 Mutation Analysis

The results of assays for DNA mutations will be reported to the Sponsor directly by the laboratory performing those assays, and will not be included in this study report.

4.0 Discussion and Conclusions

The results of this study demonstrated that SDD administered to rats in drinking water for 90 days was well tolerated. No mortality and no clinical signs of toxicity were observed during the study. The deficits in water consumption observed for rats in the 60, 170, and 520 mg/L groups were considered to be likely due to palatability problems rather than SDD toxicity. The deficit in the highest SDD treatment group was consistent with and was likely at least partly responsible for the minimal deficits in mean body weight observed for animals in the 520 mg/L group. Similar changes in water consumption and minimal reductions in body weight gain were reported by the NTP for female Fischer rats administered SDD in drinking water at concentrations of 172 and 516 mg/L for 2 years.[1,3] This is illustrated in the two tables below. For female rats in the 500-520 mg/L dose range, mean body weights at the 13-week time point in the current study, the NTP 90-day study, and the NTP 2-year were 3, 0, and 4% lower, respectively, than controls. For female rats in the 500-520 mg/L dose range, mean water consumption values at the 13-week time point in the current study, the NTP 90-day study, and the NTP 2-year were 25, 19, and 32% lower, respectively, than controls. Mean water consumption values for rats in this study were

Body Weight Comparisons Between Current Study and NTP Studies

Current Study 13-Week Time Point		NTP 90-Day Study 13-Week Time Point		NTP 2-Year Study 13-Week Time Point	
Concentration (mg/L)	% Difference from Control	Concentration (mg/L)	% Difference from Control	Concentration (mg/L)	% Difference from Control
4	-1	62.5	+8	14.3	-3
60	0	125	+3	57.3	-3
170	-2	250	+2	172	-3
520	-3	500	0	516	-4

Water Consumption Comparisons Between Current Study and NTP Studies

Current Study 13-Week Time Point		NTP 90-Day Study 13-Week Time Point		NTP 2-Year Study 13-Week Time Point	
Concentration (mg/L)	% Difference from Control	Concentration (mg/L)	% Difference from Control	Concentration (mg/L)	% Difference from Control
4	0	62.5	+13	14.3	-7
60	-12	125	-3	57.3	-12
170	-19	250	-16	172	-22
520	-25	500	-19	516	-32

Average water consumption values in the current study were higher than those reported by the NTP in their 90-day and 2-year studies. For example, the mean water consumption of rats in the 520 mg/L group at 13 weeks (15.5 g/animal/day) was 70-85% higher than the values for female rats at 13 weeks in the NTP 90-day study (9.1 g/animal/day at 500 mg/L) and 2-year study (8.4 g/animal/day at 516 mg/L). However, body weights were comparable in all three studies, suggesting that the net exposure of animals to Cr(VI) in the current study may have been somewhat higher than in the NTP studies. Water consumption values in the current study were comparable to values for rats reported in the literature.[4]

The data from this study suggest that administration of SDD to rats in drinking water may have been associated with iron deficiency, as evidenced by decreases in circulating levels of iron at concentrations of ≥60 mg/L and of iron storage in bone marrow at concentrations of 170 and 520 mg/L. These data are consistent with observations from other studies of hexavalent chromium exposure in rats, including the 90-day and 2-year studies conducted by the NTP, that suggest an effect of hexavalent chromium on iron metabolism.[1,3] Iron deficiency is usually accompanied by decreases in ferritin levels.[5] However, in the current study there were no apparent differences in ferritin levels between vehicle control and SDD-treated animals. The reason for this observation is not known. The data collected in this study do not address the mode of action by which SDD may cause iron deficiency.

In the current study, microscopic lesions were observed in the small intestine of animals in the 60, 170, and 520 mg/L groups on Day 8 and Day 91. These lesions included histiocytic cellular infiltration of the villous lamina propria (Days 8 and 91), apoptosis (Days 8 and 91), villous atrophy (Day 8), and crypt epithelial hyperplasia (Days 8 and 91). Given that the vast majority of these lesions occurred only in the groups receiving higher concentrations of SDD (60, 170, and 520 mg/L), and that the incidence and/or severity of villous atrophy, crypt epithelial hyperplasia, histiocytic cellular infiltration, and apoptosis were higher in the two highest treatment groups than in the 60 mg/L group, the microscopic lesions observed in the small intestine in the current study were considered to be a result of SDD administration. These findings are consistent with repair following an injury in the intestinal mucosa The higher frequency and increased severity of crypt hyperplasia and villous atrophy on Day 8 are

suggestive of a more acute injury. Long term administration of SDD resulted in histiocytic cellular infiltration that was observed with greater frequency and severity on Day 91.

The microscopic lesions observed in the intestine of rats in the current study were consistent with the type of non-neoplastic lesions reported by the NTP for male and female rats (histiocytic cellular infiltration) and mice (histiocytic cellular infiltration and diffuse epithelial hyperplasia) administered SDD in drinking water for either 90 days or 2 years.[1,3] As in the current study, the NTP reported an increased incidence and severity of histiocytic cellular infiltration in the duodenum of male and female rats administered SDD for 90 days or 2 years, although in general, intestinal lesions were more severe in the current study than in the studies reported by the NTP. In the previous studies, histiocytic cellular infiltration was observed in the duodenum of rats and in the duodenum and jejunum of mice. Diffuse or focal epithelial hyperplasia was observed in the duodenum and jejunum of mice. In the current rat study, the intestinal lesions were more similar to lesions observed in the previous mouse study than to the lesions observed in the previous rat studies. The reason for this discrepancy in the types of intestinal lesions observed in the current study versus the previous study is unknown. No microscopic lesions were reported in the oral mucosa of rats in the current study; these results are similar to those reported by the NTP for rats administered SDD for 90 days. However, an increased incidence of carcinoma of the oral mucosa was observed for female rats administered SDD for 2 years and was considered to be clear evidence of carcinogenic activity of hexavalent chromium in rats.[1]

An increase in intracellular free radicals leading to oxidative stress and DNA damage is one of the known mechanisms by which compounds can exert carcinogenic activity. It has been suggested that the DNA-damaging effects of hexavalent chromium may be primarily related to reduction of Cr(VI) to Cr(III), Cr(V), and Cr(IV). One proposed mechanism involves formation of DNA-Cr adducts via binding of reduced chromium directly to DNA. Another involves formation of highly reactive free radicals as by-products of the reduction of Cr(VI) to Cr(III).[6] The production of lung tumors by hexavalent chromium after inhalation exposure is believed to occur at least in part due to such free radical production.[7]

Changes in levels of various markers of oxidative stress, including tissue levels of 8-OHdG and 8-isoprostane; plasma and tissue levels of a panel of cytokines/chemokines; tissue levels of DNA-Cr adducts; and plasma and tissue levels of reduced and oxidized glutathione were evaluated in this study. The data for adduct formation and changes in GSH/GSSG ratio will be reported to the Sponsor by the laboratories performing those analyses, separately from this report. It is noteworthy that no apparent changes in 8-OHdG, 8-isoprostane, or pro-inflammatory cytokine/chemokine levels were observed in the oral cavity of rats administered SDD in this study. This observation is consistent with the lack of microscopic changes in the oral cavity at 90 days. Similarly, the apparent increase in levels of the pro-inflammatory cytokine IL-1α in the duodenum at SDD concentrations ≥60 mg/L is consistent with the histiocytic cellular infiltration observed in this tissue at the same concentrations. The relevance of the apparent decreases in IL-12p70 in serum at the same concentrations and the decrease in leptin in serum at 520 mg/L is not known.

In summary, the microscopic results for oral cavity and duodenum that were observed in this study were consistent with those seen in other studies where rats were administered hexavalent chromium in drinking water. Due to the microscopic changes observed in the duodenum and jejunum of animals in the 60 mg/L group on Day 91, as well as observed effects on water consumption and serum iron in the same dose group, the no observed effect level (NOEL) was determined to be 4 mg/L SDD under the conditions of this study.

5.0 Record Archives

All raw data pertaining to the conduct of this study, and all samples/specimens generated in this study, will be stored at Southern Research for up to 1 year after the issuance of the draft report. After 1 year, or at any time prior to the completion of that year if the Sponsor's Monitor so directs, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor’s designated archival facility. The Sponsor must approve the final disposition of all raw data and samples/specimens generated in this study. The original final report will be retained in the central Archives at Southern Research.

6.0 References

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3. National Toxicology Program (NTP) (2007). NTP Report on 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. NTP TOX 72. NIH Publication No. 07-5964. National Institutes of Health.

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5. Centers for Disease Control and Prevention (1998). Recommendations to prevent and control iron deficiency in the United States. *Morb. Mortal. Wkly. Rep.* **47(RR-3),** 1-29.

6. Cohen, M.D., Kargacin, B., Klein, C.B., and Costa, M. (1993). Mechanisms of chromium carcinogenicity and toxicity. *Crit. Rev. Toxicol.* **23(3),** 255-281.

7. Dayan, A.D. and Paine, A.J. (2001). Mechanisms of chromium toxicity, carcinogenicity and allergenicity: Review of the literature from 1985 to 2000. *Human Exp. Toxicol.* **20(9),** 439–451.

7.0 Comments on Study Data

The following is a list of protocol and/or SOP deviations occurring during this study that have not been mentioned elsewhere in this report. Unless otherwise noted, the Study Director determined that no deviation had an adverse impact on the outcome of the study. The following may also list incidents in recording data and general comments on the study data.

Protocol Section 8.2 required that the environmental conditions in the animal room be maintained at 69-75 °C and 35-65% relative humidity. Minor excursions beyond these limits occurred on several occasions. These excursions were determined to have had no effect on animal health.

Protocol Section 9.10 required that prior to freezing the length of the intestinal segments collected for total chromium and iron analysis be recorded. The length of jejunum for animal 2F123 and length of ileum for animal 5F346 were not recorded.

Protocol Section 9.10 required that samples of plasma and RBC collected for total chromium and iron analysis be stored at approximately -20 °C. Upon instructions from the study director, these samples were instead stored in the -80 °C freezer.

The Specimen Collection Log Sheet used for serum iron analysis samples is not present in the study records. This form contained the following information: anesthetic used, collection site, type of blood tubes used, animal ID numbers, times of collection, and initials of the person performing the collection. The fact that the samples were collected, the date, and the identity of the animals used are documented in other places in the data.

Table 1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Correlation of Animal Number, Cage Number, SDD Concentration, and Analysis Group

Animal Numbers	**Cage Number**	**Dose Level (mg/L)**	**Analysis Group**
1 – 5	1	0	Gene Expression Analysis, Day 8
6 – 10	2	0	Gene Expression Analysis, Day 8
11 – 15	3	0	Toxicology/Histology Day 91, Cohort 1
16 – 20	4	0	Toxicology/Histology Day 91, Cohort 2
21 – 25	5	0	Biochemical Evaluations, Day 91, Cohort 1
26 – 30	6	0	Biochemical Evaluations, Day 91, Cohort 1
31 – 35	7	0	Biochemical Evaluations, Day 91, Cohort 2
36 – 40	8	0	Gene Expression Analysis, Day 91
41 – 45	9	0	Gene Expression Analysis, Day 91
46 – 50	10	0	Chromium and Iron Analysis, Day 91
51 – 55	11	0	Mutation Analysis, Day 91, Cohort 1
56 – 60	12	0	Mutation Analysis, Day 91, Cohort 2
61 – 65	13	0	Biochemical Evaluations, Day 8
66 – 70	14	0	Biochemical Evaluations, Day 8
71 – 75	15	0	Toxicology/Histology Day 8
76 – 80	16	0.3	Gene Expression Analysis, Day 8
81 – 85	17	0.3	Gene Expression Analysis, Day 8
86 – 90	18	0.3	Toxicology/Histology Day 91, Cohort 1
91 – 95	19	0.3	Toxicology/Histology Day 91, Cohort 2
96 – 100	20	0.3	Biochemical Evaluations, Day 91, Cohort 1
101 – 105	21	0.3	Biochemical Evaluations, Day 91, Cohort 1
106 – 110	22	0.3	Biochemical Evaluations, Day 91, Cohort 2
111 – 115	23	0.3	Gene Expression Analysis, Day 91
116 – 120	24	0.3	Gene Expression Analysis, Day 91
121 – 125	25	0.3	Chromium and Iron Analysis, Day 91
126 – 130	26	0.3	Mutation Analysis, Day 91, Cohort 1
131 – 135	27	0.3	Mutation Analysis, Day 91, Cohort 2
136 – 140	28	0.3	Biochemical Evaluations, Day 8
141 – 145	29	0.3	Biochemical Evaluations, Day 8
146 – 150	30	0.3	Toxicology/Histology Day 8
151 – 155	31	4	Gene Expression Analysis, Day 8
156 – 160	32	4	Gene Expression Analysis, Day 8
161 – 165	33	4	Toxicology/Histology Day 91, Cohort 1
166 – 170	34	4	Toxicology/Histology Day 91, Cohort 2
171 – 175	35	4	Biochemical Evaluations, Day 91, Cohort 1
176 – 180	36	4	Biochemical Evaluations, Day 91, Cohort 1
181 – 185	37	4	Biochemical Evaluations, Day 91, Cohort 2
186 – 190	38	4	Gene Expression Analysis, Day 91

Table 1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Correlation of Animal Number, Cage Number, SDD Concentration, and Analysis Group

Animal Numbers	**Cage Number**	**Dose Level (mg/L)**	**Analysis Group**
191 – 195	39	4	Gene Expression Analysis, Day 91
196 – 200	40	4	Chromium and Iron Analysis, Day 91
201 – 205	41	4	Mutation Analysis, Day 91, Cohort 1
206 – 210	42	4	Mutation Analysis, Day 91, Cohort 2
211 – 215	43	4	Biochemical Evaluations, Day 8
216 – 220	44	4	Biochemical Evaluations, Day 8
221 – 225	45	4	Toxicology/Histology Day 8
226 – 230	46	60	Gene Expression Analysis, Day 8
231 – 235	47	60	Gene Expression Analysis, Day 8
236 – 240	48	60	Toxicology/Histology Day 91, Cohort 1
241 – 245	49	60	Toxicology/Histology Day 91, Cohort 2
246 – 250	50	60	Biochemical Evaluations, Day 91, Cohort 1
251 – 255	51	60	Biochemical Evaluations, Day 91, Cohort 1
256 – 260	52	60	Biochemical Evaluations, Day 91, Cohort 2
261 – 265	53	60	Gene Expression Analysis, Day 91
266 – 270	54	60	Gene Expression Analysis, Day 91
271 – 275	55	60	Chromium and Iron Analysis, Day 91
276 – 280	56	60	Mutation Analysis, Day 91, Cohort 1
281 – 285	57	60	Mutation Analysis, Day 91, Cohort 2
286 – 290	58	60	Biochemical Evaluations, Day 8
291 – 295	59	60	Biochemical Evaluations, Day 8
296 – 300	60	60	Toxicology/Histology Day 8
301 – 305	61	170	Gene Expression Analysis, Day 8
306 – 310	62	170	Gene Expression Analysis, Day 8
311 – 315	63	170	Toxicology/Histology Day 91, Cohort 1
316 – 320	64	170	Toxicology/Histology Day 91, Cohort 2
321 – 325	65	170	Biochemical Evaluations, Day 91, Cohort 1
326 – 330	66	170	Biochemical Evaluations, Day 91, Cohort 1
331 – 335	67	170	Biochemical Evaluations, Day 91, Cohort 2
336 – 340	68	170	Gene Expression Analysis, Day 91
341 – 345	69	170	Gene Expression Analysis, Day 91
346 – 350	70	170	Chromium and Iron Analysis, Day 91
351 – 355	71	170	Mutation Analysis, Day 91, Cohort 1
356 – 360	72	170	Mutation Analysis, Day 91, Cohort 2
361 – 365	73	170	Biochemical Evaluations, Day 8
366 – 370	74	170	Biochemical Evaluations, Day 8
371 – 375	75	170	Toxicology/Histology Day 8
376 – 380	76	520	Gene Expression Analysis, Day 8

Table 1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Correlation of Animal Number, Cage Number, SDD Concentration, and Analysis Group

Animal Numbers	**Cage Number**	**Dose Level (mg/L)**	**Analysis Group**
381 – 385	77	520	Gene Expression Analysis, Day 8
386 – 390	78	520	Toxicology/Histology Day 91, Cohort 1
391 – 395	79	520	Toxicology/Histology Day 91, Cohort 2
396 – 400	80	520	Biochemical Evaluations, Day 91, Cohort 1
401 – 405	81	520	Biochemical Evaluations, Day 91, Cohort 1
406 – 410	82	520	Biochemical Evaluations, Day 91, Cohort 2
411 – 415	83	520	Gene Expression Analysis, Day 91
416 – 420	84	520	Gene Expression Analysis, Day 91
421 – 425	85	520	Chromium and Iron Analysis, Day 91
426 – 430	86	520	Mutation Analysis, Day 91, Cohort 1
431 – 435	87	520	Mutation Analysis, Day 91, Cohort 2
436 – 440	88	520	Biochemical Evaluations, Day 8
441 – 445	89	520	Biochemical Evaluations, Day 8
446 – 450	90	520	Toxicology/Histology Day 8

Table 2

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Weights of Tissues Collected for Assay of 8-OHdG, 8-Isoprostane, and Cytokine Levels:

Tissue Weight (g)

Animal ID	Duodenum	Oral Cavity
1F31	0.3640	0.0895
1F32	0.3080	0.0757
1F33	0.4419	0.0480
1F34	0.2453	0.0540
1F35	0.3151	0.0851
Mean	**0.3349**	**0.0705**
S.D.	**0.0732**	**0.0186**

Animal ID	Duodenum	Oral Cavity
2F106	0.3803	0.0669
2F107	0.4196	0.0873
2F108	0.4419	0.0972
2F109	0.3330	0.1115
2F110	0.4669	0.0742
Mean	**0.4083**	**0.0874**
S.D.	**0.0528**	**0.0178**

Animal ID	Duodenum	Oral Cavity
3F181	0.4049	0.0858
3F182	0.4166	0.0637
3F183	0.3784	0.0925
3F184	0.4199	0.0502
3F185	0.3852	0.1234
Mean	**0.4010**	**0.0831**
S.D.	**0.0185**	**0.0282**

Animal ID	Duodenum	Oral Cavity
4F256	0.4931	0.0963
4F257	0.4348	0.0681
4F258	0.4953	0.0965
4F259	0.3692	0.0896
4F260	0.4072	0.0664
Mean	**0.4399**	**0.0834**
S.D.	**0.0548**	**0.0150**

Animal ID	Duodenum	Oral Cavity
5F332	0.4092	0.1125
5F332	0.4971	0.0583
5F333	0.4037	0.1015
5F334	0.3616	0.0816
5F335	0.5038	0.0583
Mean	**0.4351**	**0.0824**
S.D.	**0.0625**	**0.0247**

Animal ID	Duodenum	Oral Cavity
6F406	0.4197	0.0602
6F407	0.4738	0.1039
6F408	0.4188	0.0721
6F409	0.4663	0.0527
6F410	0.4579	0.0789
Mean	**0.4473**	**0.0736**
S.D.	**0.0262**	**0.0198**

Table 3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Sodium Dichromate Dihydrate Dose Concentration Analysis

Date Mixed	Date Analyzed	Concentration (mg/L)		% High/Low of Theoretical
		Theoretical	Actual	
7/21/10	7/22/10	0	0.01	NA
7/21/10	7/22/10	0.3	0.30	101.23
7/21/10	7/22/10	4	3.93	98.13
7/21/10	7/22/10	60	57.59	95.98
7/21/10	7/22/10	170	155.86	91.68
7/21/10	7/22/10	520	472.73	90.91
8/16/10	8/17/10	0	0.02	NA
8/16/10	8/17/10	0.3	0.28	93.02
8/16/10	8/17/10	4	3.95	98.84
8/16/10	8/17/10	60	57.87	96.46
8/16/10	8/17/10	170	159.87	94.04
8/16/10	8/17/10	520	504.24	96.97
9/13/10	9/14/10	0	0.02	NA
9/13/10	9/14/10	0.3	0.30	99.32
9/13/10	9/14/10	4	3.87	96.69
9/13/10	9/14/10	60	55.30	92.16
9/13/10	9/14/10	170	159.01	93.53
9/13/10	9/14/10	520	484.19	93.11
10/27/10	10/28/10	0	0.02	NA
10/27/10	10/28/10	0.3	0.29	97.41
10/27/10	10/28/10	4	3.90	97.41
10/27/10	10/28/10	60	59.02	98.37
10/27/10	10/28/10	170	166.74	98.08
10/27/10	10/28/10	520	521.43	100.28

NA = Not applicable
Note: Values reported were calculated based on nontruncated raw data; therefore, some values may not be reproducible when calculated from rounded values presented in this table.

Table 4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Clinical Observations

Day numbers relative to Start Date

Group	Sex	Clinical Sign	1	8	15	22	29	36	43	50	57	64	71
1	f	ANIMALS ALIVE	75	75	50	50	50	50	50	50	50	50	50
		ANIMALS NORMAL	75	75	50	50	50	49	47	48	49	47	48
		Alopecia	.	.	.	.	.	.	1	1	1	1	1
		Discharge	.	.	.	.	.	1	2	1	.	2	.
		Hyperexcitable	.	.	.	.	.	.	.	.	.	.	1
		Scheduled euthanasia	.	25	.	.	.	.	.	.	.	.	.
2	f	ANIMALS ALIVE	75	75	50	50	50	50	50	50	50	50	50
		ANIMALS NORMAL	75	75	50	50	50	50	49	48	48	44	44
		Alopecia	.	.	.	.	.	.	.	2	2	4	4
		Discharge	.	.	.	.	.	.	1	.	.	2	2
		Scheduled euthanasia	.	25	.	.	.	.	.	.	.	.	.
3	f	ANIMALS ALIVE	75	75	50	50	50	50	50	50	50	50	50
		ANIMALS NORMAL	75	75	50	50	50	48	48	48	47	45	48
		Alopecia	.	.	.	.	.	2	2	2	2	5	2
		Discharge	.	.	.	.	.	.	.	.	1	.	.
		Piloerection	.	.	.	.	.	.	.	.	.	.	.
		Scheduled euthanasia	.	25	.	.	.	.	.	.	.	.	.
4	f	ANIMALS ALIVE	75	75	50	50	50	50	50	50	50	50	50
		ANIMALS NORMAL	75	75	50	50	50	50	50	50	50	49	49
		Alopecia	.	.	.	.	.	.	.	.	.	.	.
		Discharge	.	.	.	.	.	.	.	.	.	1	1
		Scheduled euthanasia	.	25	.	.	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Clinical Observations

Day numbers relative to Start Date

Group	Sex	Clinical Sign	7 8	8 5	9 1
1	f	ANIMALS ALIVE	50	50	50
		ANIMALS NORMAL	45	45	46
		Alopecia	4	4	4
		Discharge	1	1	.
		Hyperexcitable	.	.	.
		Scheduled euthanasia	.	.	50
2	f	ANIMALS ALIVE	50	50	50
		ANIMALS NORMAL	44	46	47
		Alopecia	5	4	3
		Discharge	1	.	.
		Scheduled euthanasia	.	.	50
3	f	ANIMALS ALIVE	50	50	50
		ANIMALS NORMAL	40	41	37
		Alopecia	7	8	8
		Discharge	4	2	2
		Piloerection	.	.	5
		Scheduled euthanasia	.	.	50
4	f	ANIMALS ALIVE	50	50	50
		ANIMALS NORMAL	48	49	50
		Alopecia	1	.	.
		Discharge	1	1	.
		Scheduled euthanasia	.	.	50

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Clinical Observations

Day numbers relative to Start Date

Group	Sex	Clinical Sign	1	8	15	22	29	36	43	50	57	64	71
5	f	ANIMALS ALIVE	75	75	50	50	50	50	50	50	50	50	50
		ANIMALS NORMAL	75	75	49	49	47	45	45	46	47	45	37
		Alopecia	.	.	1	1	3	3	3	3	3	5	11
		Discharge	.	.	.	.	.	2	2	1	.	1	3
		Scheduled euthanasia	.	25	.	.	.	.	.	.	.	.	.
6	f	ANIMALS ALIVE	75	75	50	50	50	50	50	50	50	50	50
		ANIMALS NORMAL	75	75	50	50	50	49	47	49	47	48	50
		Alopecia	.	.	.	.	.	.	.	.	.	.	.
		Discharge	.	.	.	.	.	1	3	1	3	2	.
		Discoloration	.	.	.	.	.	.	.	.	.	.	.
		Scheduled euthanasia	.	25	.	.	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Clinical Observations

Day numbers relative to Start Date

Group	Sex	Clinical Sign	7 8	8 5	9 1
5	f	ANIMALS ALIVE	50	50	50
		ANIMALS NORMAL	31	35	35
		Alopecia	15	14	14
		Discharge	5	2	2
		Scheduled euthanasia	.	.	50
6	f	ANIMALS ALIVE	50	50	50
		ANIMALS NORMAL	48	45	45
		Alopecia	.	1	1
		Discharge	2	4	3
		Discoloration	.	.	1
		Scheduled euthanasia	.	.	50

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Body Weights (grams)

Day numbers relative to Start Date

Group	Sex		Week -1	1	8	15	22	29	36	43
1	f	Mean	82.84	104.89	115.32	123.57	138.61	145.45	150.65	154.57
		S.D.	7.48	8.32	8.74	11.04	6.24	6.04	5.67	6.25
		N	75	75	75	50	50	50	50	50
2	f	Mean	82.85	105.34	115.49	124.46	138.04	145.18	149.29	154.03
		S.D.	7.50	9.09	10.30	11.59	6.91	7.57	7.58	8.24
		N	75	75	75	50	50	50	50	50
3	f	Mean	82.86	105.53	115.41	123.31	138.86	144.85	150.94	155.44
		S.D.	7.51	7.74	9.15	13.09	6.66	6.37	6.29	6.00
		N	75	75	75	50	50	50	50	50
4	f	Mean	82.83	105.14	116.00	127.01	138.46	145.80	150.65	154.62
		S.D.	7.47	9.36	10.76	9.52	5.65	5.58	5.18	5.93
		N	75	75	75	50	50	50	50	50
5	f	Mean	82.82	105.63	114.88	122.69	136.54	143.73	148.28	151.99
		S.D.	7.44	8.15	7.89	11.19	6.40	6.82	7.00	6.79
		N	75	75	75	50	50	50	50	50
6	f	Mean	82.89	104.93	113.75	123.09	135.39	142.82	147.24*	150.54*
		S.D.	7.49	8.75	8.31	7.96	6.84	7.37	7.38	7.40
		N	75	75	75	50	50	50	50	50

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex		50	57	64	71	78	85	91
1	f	Mean	155.97	161.32	163.50	168.33	169.53	173.06	177.81
		S.D.	9.53	7.39	6.93	6.75	7.33	7.07	7.44
		N	50	50	50	50	50	50	50
2	f	Mean	157.87	160.76	163.15	167.81	168.87	172.68	176.73
		S.D.	7.61	8.07	8.17	9.15	8.83	9.23	9.26
		N	50	50	50	50	50	50	50
3	f	Mean	159.05	162.94	164.94	170.34	170.85	174.58	176.36
		S.D.	7.09	7.12	7.18	7.88	7.65	8.07	10.85
		N	50	50	50	50	50	50	50
4	f	Mean	158.60	162.34	164.93	169.04	170.03	174.27	177.23
		S.D.	6.25	6.68	6.45	6.14	6.13	6.48	6.75
		N	50	50	50	50	50	50	50
5	f	Mean	155.99	159.14	161.60	165.64	164.67*	170.42	173.73
		S.D.	6.62	7.70	7.32	7.93	9.33	8.10	8.13
		N	50	50	50	50	50	50	50
6	f	Mean	154.36	157.92	160.50	164.69	165.82	168.78*	171.93*
		S.D.	7.26	8.31	7.93	8.38	8.85	9.35	8.92
		N	50	50	50	50	50	50	50

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 6

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Food Consumption (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 76	71 / 77	71 / 78	76 / 78	77 / 78	78 / 85
1	f	Mean	10.2	10.6	11.1	10.5	10.1	10.1	9.5	10.0	9.9	9.7	10.4	9.7	9.5	10.8	10.0	9.8
		S.D.	1.0	1.6	0.8	1.1	0.8	0.5	0.9	0.6	0.5	0.4	.	.	0.3	.	.	0.6
		N	15	10	10	9	10	10	10	10	10	9	1	1	8	1	1	10
2	f	Mean	10.3	11.0	11.0	10.6	10.2	10.1	9.7	9.8	9.9	9.9	10.6	9.4	9.5	9.8	11.8	9.8
		S.D.	0.9	1.4	1.0	1.1	0.2	0.5	0.2	0.6	0.5	0.5	.	.	0.5	.	.	0.7
		N	14	10	10	10	10	10	10	10	10	10	1	1	8	1	1	10
3	f	Mean	10.0	10.6	10.9	10.4	10.2	9.9	9.7	9.9	9.8	9.8	10.2	9.5	9.5	10.5	8.6	9.7
		S.D.	0.8	1.9	0.8	0.8	0.4	0.9	0.4	0.4	0.4	0.5	.	.	0.5	.	.	0.6
		N	14	10	10	8	9	10	10	10	10	10	1	1	8	1	1	10
4	f	Mean	10.2	11.0	10.3	10.5	10.3	10.5	10.0	10.0	9.9	10.0	9.8	9.6	9.5	10.5	11.0	9.8
		S.D.	1.2	1.2	1.1	0.7	0.3	1.2	0.5	0.5	0.4	0.7	.	.	0.4	.	.	0.5
		N	13	9	10	10	10	10	10	10	9	9	1	1	8	1	1	10
5	f	Mean	9.7	10.3	10.5	10.6	10.3	10.0	9.9	9.7	9.8	9.8	9.8	9.5	9.5	9.7	9.8	9.8
		S.D.	0.8	1.7	1.1	1.3	0.5	0.4	0.7	0.3	0.4	0.6	.	.	0.5	.	.	0.3
		N	14	10	10	10	10	10	10	10	9	10	1	1	7	1	1	9
6	f	Mean	9.5	11.2	11.7	10.8	11.0	10.4	10.4*	10.2	10.7*	10.2	10.0	10.3	9.9	10.1	10.6	9.8
		S.D.	0.8	0.6	2.0	0.6	1.3	0.4	0.5	0.4	0.6	0.4	.	.	0.5	.	.	1.0
		N	12	9	9	10	9	10	9	9	10	8	1	1	7	1	1	9

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 6

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Food Consumption (grams/animal/day)

Day numbers relative to Start Date

Group	Sex		From: 85 To: 91
1	f	Mean	9.9
		S.D.	0.4
		N	10
2	f	Mean	10.0
		S.D.	0.5
		N	10
3	f	Mean	9.3
		S.D.	1.3
		N	10
4	f	Mean	9.9
		S.D.	0.9
		N	7
5	f	Mean	9.8
		S.D.	0.6
		N	9
6	f	Mean	10.4
		S.D.	0.8
		N	9

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Water Consumption (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 78	78 / 85	85 / 91
1	f	Mean	23.6	25.7	24.5	25.0	23.8	23.7	22.9	22.2	21.7	21.3	20.8	21.0	20.7
		S.D.	1.4	6.2	1.4	5.4	1.0	2.8	3.8	2.0	1.6	1.6	1.0	1.3	0.7
		N	14	10	10	10	10	10	10	10	10	10	10	9	10
2	f	Mean	24.6	24.0	23.7	22.7	22.9	22.3	21.6	23.4	21.9	21.5	20.3	20.8	20.3
		S.D.	1.6	2.3	1.5	1.3	1.5	1.0	1.6	4.8	1.9	1.5	2.0	1.7	1.2
		N	15	10	10	10	10	10	10	9	10	10	10	10	9
3	f	Mean	23.7	23.3	25.0	24.3	23.3	22.6	21.8	22.6	22.1	21.9	20.9	21.6	19.6
		S.D.	2.1	3.3	1.5	3.3	1.4	1.7	1.5	1.6	1.8	1.8	1.5	1.3	3.2
		N	15	10	9	10	10	10	10	10	10	10	9	10	9
4	f	Mean	23.4	22.8	25.5	21.6*	21.5*	20.2*	20.2*	19.8	19.9	19.4	19.0*	19.3	18.3*
		S.D.	2.0	2.2	10.4	1.1	1.3	1.0	0.9	0.8	1.2	1.2	1.5	0.9	0.8
		N	15	10	9	10	10	10	9	9	10	10	10	10	10
5	f	Mean	20.2*	18.8*	19.5*	18.4*	18.2*	17.8*	17.4*	17.2*	17.1*	16.4*	16.0*	17.3*	16.7*
		S.D.	2.3	2.6	1.4	1.2	1.0	0.8	0.7	0.8	1.2	0.7	0.6	1.9	1.3
		N	15	10	10	10	10	10	10	10	10	10	9	10	10
6	f	Mean	17.7*	16.8*	17.2*	17.8*	18.3*	16.3*	16.2*	16.3*	17.1*	16.8*	15.4*	14.9*	15.5*
		S.D.	4.2	1.5	1.1	2.9	3.2	1.0	0.9	1.1	3.6	4.0	1.4	1.4	2.5
		N	15	10	10	10	10	10	9	10	10	10	9	10	10

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Water consumption units are mL/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table 8

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Organ Weights (g) and Intestinal Segment Lengths (cm):
Samples Collected for Total Cr and Fe Analysis

Animal ID	Oral Mucosa (g)	Bone (g)	Spleen (g)	Kidney (g)	Liver (g)	Glandular Stomach (g)	Duodenum (g)	Jejunum (g)	Ileum (g)	Duodenum (cm)	Jejunum (cm)	Ileum (cm)
1F46	0.0767	1.1107	0.3231	1.1267	5.3392	0.7305	0.3216	3.2881	0.2082	5.4	66.5	6.5
1F47	0.0577	1.0608	0.4249	1.1334	5.5993	0.8399	0.4561	3.1230	0.2393	5.4	66.5	6.4
1F48	0.0853	1.1134	0.3782	1.1590	5.6804	0.7157	0.4436	2.7908	0.2786	5.2	66.6	5.2
1F49	0.0825	1.1045	0.3732	1.2180	5.9818	0.7578	0.3519	2.9601	0.2556	3.7	69.5	6.0
1F50	0.0712	1.0275	0.3844	1.1048	5.6127	0.6004	0.4365	2.9343	0.1668	6.3	64.6	6.4
Group Mean	**0.0747**	**1.0834**	**0.3768**	**1.1484**	**5.6427**	**0.7289**	**0.4019**	**3.0193**	**0.2297**	**5.2**	**66.7**	**6.1**
SD	**0.0109**	**0.0378**	**0.0363**	**0.0435**	**0.2298**	**0.0864**	**0.0609**	**0.1910**	**0.0435**	**0.9**	**1.8**	**0.5**
2F121	0.0513	1.2912	0.4098	1.3093	5.4542	0.6965	0.5834	2.9785	0.2007	6.0	66.0	6.0
2F122	0.0727	1.0677	0.3853	1.2283	5.4995	0.7185	0.5106	3.0304	0.3039	6.4	63.0	8.0
2F123	0.0871	0.9308	0.3821	1.1419	5.1529	0.7621	0.4149	3.2371	0.2748	5.2	---	6.4
2F124	0.0663	1.3578	0.3649	1.3451	5.9434	0.7177	0.5263	2.4524	0.2281	6.0	66.0	6.0
2F125	0.0892	1.1925	0.3505	1.2017	5.4445	0.8695	0.4950	2.9483	0.2460	6.4	71.9	6.8
Group Mean	**0.0733**	**1.1680**	**0.3785**	**1.2453**	**5.4989**	**0.7529**	**0.5060**	**2.9293**	**0.2507**	**6.0**	**66.7**	**6.6**
SD	**0.0156**	**0.1718**	**0.0224**	**0.0821**	**0.2838**	**0.0694**	**0.0609**	**0.2895**	**0.0401**	**0.5**	**3.7**	**0.8**

Table 8

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Organ Weights (g) and Intestinal Segment Lengths (cm)
Samples Collected for Total Cr and Fe Analysis

Animal ID	Oral Mucosa (g)	Bone (g)	Spleen (g)	Kidney (g)	Liver (g)	Glandular Stomach (g)	Duodenum (g)	Jejunum (g)	Ileum (g)	Duodenum (cm)	Jejunum (cm)	Ileum (cm)
3F196	0.0458	0.8970	0.3999	1.1379	5.6278	0.4968	0.2928	2.9630	0.2542	5.8	67.5	6.4
3F197	0.0527	0.8574	0.3635	1.0871	5.4265	0.6568	0.3737	2.8291	0.2139	4.2	91.0	6.4
3F198	0.0645	1.4217	0.3975	1.1900	5.9447	0.6670	0.5123	2.6301	0.2286	6.0	65.0	6.0
3F199	0.0804	1.2231	0.3948	1.2437	5.7909	0.8430	0.5128	2.8560	0.3214	6.5	70.0	8.7
3F200	0.0604	0.9955	0.3629	1.1244	5.2333	0.7295	0.3742	1.9578	0.3324	5.6	69.6	7.3
Group Mean	**0.0608**	**1.0789**	**0.3837**	**1.1566**	**5.6046**	**0.6786**	**0.4132**	**2.6472**	**0.2701**	**5.6**	**72.6**	**7.0**
SD	**0.0131**	**0.2384**	**0.0188**	**0.0611**	**0.2831**	**0.1258**	**0.0966**	**0.4037**	**0.0540**	**0.9**	**10.5**	**1.1**
4F271	0.0742	1.4117	0.3796	1.1476	5.6763	0.5990	0.4443	3.0315	0.1757	5.5	70.0	5.5
4F272	0.0913	1.0458	0.3659	1.3251	5.6735	0.6764	0.3670	2.9269	0.2569	6.0	63.5	6.4
4F273	0.0718	1.1806	0.4263	1.2604	6.2228	0.8176	0.4529	2.6741	0.3782	5.8	68.0	10.0
4F274	0.0925	1.1937	0.4103	1.1907	5.5828	0.7011	0.4565	3.2016	0.2502	5.0	68.0	5.0
4F275	0.0729	1.1102	0.4175	1.1738	5.9519	0.4701	0.4194	2.8505	0.2754	5.8	64.5	6.4
Group Mean	**0.0805**	**1.1884**	**0.3999**	**1.2195**	**5.8215**	**0.6528**	**0.4280**	**2.9369**	**0.2673**	**5.6**	**66.8**	**6.7**
SD	**0.0104**	**0.1382**	**0.0259**	**0.0723**	**0.2636**	**0.1288**	**0.0371**	**0.1973**	**0.0727**	**0.4**	**2.7**	**2.0**

Table 8

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Organ Weights (g) and Intestinal Segment Lengths (cm)
Samples Collected for Total Cr and Fe Analysis

Animal ID	Oral Mucosa (g)	Bone (g)	Spleen (g)	Kidney (g)	Liver (g)	Glandular Stomach (g)	Duodenum (g)	Jejunum (g)	Ileum (g)	Duodenum (cm)	Jejunum (cm)	Ileum (cm)
5F346	0.0730	1.0157	0.4333	1.1701	5.5919	0.6829	0.3858	2.8863	0.2468	4.4	72.0	---
5F347	0.0754	1.2393	0.3566	1.2710	5.6811	0.7704	0.4909	2.8156	0.1715	5.0	70.5	5.0
5F348	0.0768	0.8649	0.3803	1.2407	5.3212	0.5793	0.4418	2.9322	0.2343	6.4	67.5	6.4
5F349	0.0835	1.1252	0.4010	1.2642	5.8793	0.7634	0.5098	2.8284	0.2939	6.0	77.3	6.0
5F350	0.0841	1.3098	0.3969	1.2332	5.5987	0.7403	0.5100	3.2194	0.1943	6.0	64.5	6.0
Group Mean	**0.0786**	**1.1110**	**0.3936**	**1.2358**	**5.6144**	**0.7073**	**0.4677**	**2.9364**	**0.2282**	**5.6**	**70.4**	**5.9**
SD	**0.0050**	**0.1774**	**0.0282**	**0.0400**	**0.2008**	**0.0794**	**0.0536**	**0.1650**	**0.0476**	**0.8**	**4.8**	**0.6**
6F421	0.0947	1.0000	0.4180	1.3269	5.6881	0.6729	0.4885	3.0519	0.2782	5.9	66.5	6.4
6F422	0.0745	1.1561	0.4061	1.2446	5.6293	0.6756	0.6153	3.8208	0.2612	5.1	68.0	6.1
6F423	0.1139	1.1286	0.3791	1.1791	5.3633	0.6009	0.5644	2.3142	0.2275	6.1	70.0	6.0
6F424	0.0723	1.1461	0.5706	1.2952	6.4057	0.8036	0.5878	3.0324	0.2369	5.2	66.5	6.1
6F425	0.0691	0.9127	0.3368	1.2321	4.6717	0.7481	0.5430	3.0416	0.2889	6.1	60.0	6.4
Group Mean	**0.0849**	**1.0687**	**0.4221**	**1.2556**	**5.5516**	**0.7002**	**0.5598**	**3.0522**	**0.2585**	**5.7**	**66.2**	**6.2**
SD	**0.0191**	**0.1076**	**0.0887**	**0.0574**	**0.6253**	**0.0778**	**0.0481**	**0.5329**	**0.0262**	**0.5**	**3.8**	**0.2**

Appendix A

Operational Protocol and Amendments

Study Protocol:

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Southern Research Institute Study No: 13026.01.02

July 21, 2010

IACUC No.: 10-01-003B Approval Date: 1/13/10

SOUTHERN RESEARCH

Legendary Discoveries. Leading Innovation.

1.0 SPONSOR REPRESENTATIVE AND CONTACTS:

Sponsor:

American Chemistry Council
1300 Wilson Avenue
Arlington, VA 22209

Sponsor's Representative:

Mark A. Harris, Ph.D.
ToxStrategies, Inc.
23501 Cinco Ranch Blvd.
Suite G265
Katy, TX 77494
E-mail: mharris@toxstrategies.com
Tel: 281-712-2062 Ext 2001

Test Article:

Sodium dichromate dihydrate (SDD)

2.0 TITLE:

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

3.0 OBJECTIVE:

The objective of this study is to evaluate the toxicity and potential mechanisms of action of sodium dichromate dihydrate (SDD) administered in drinking water to rats for 90 days.

4.0 TESTING LABORATORY:

Drug Development Division
Southern Research Institute (Southern Research)
2000 Ninth Avenue South 35205
P.O. Box 55305
Birmingham, AL 35255-5305

5.0 KEY STUDY DATES:

Event	Sequence	Dates
Day 1 of Dosing	Toxicology/Histology Groups (Day 8 Necropsy, 5/group) Toxicology/Histology Groups (Day 91 Necropsy, 5/group) Toxicology/Histology Groups (Day 91 Necropsy, 5/group) Biochemical Evaluation Groups (Day 8, 10/group) Biochemical Evaluation Groups (Day 91, 10/group) Biochemical Evaluation Groups (Day 91, 5/group) Gene Expression Groups (Day 8, 10/group) Gene Expression Groups (Day 91, 10/group) Mutation Analysis Groups (Day 91, 5/group) Mutation Analysis Groups (Day 91, 5/group) Total Chromium and Iron Analyses (Day 91, 5/group)	8/27/10 8/3/10 8/4/10 8/26/10 8/5/10 8/6/10 8/2/10 8/7/10 8/24/10 8/25/10 8/10/10
Collection of Samples for Biochemical Analysis	Day 8 Day 91	9/2/10 11/3-4/10
Collection of Samples for Gene Expression Analysis	Day 8 Day 91	8/9/10 11/5/10
Collection of Samples for Mutation Analysis	Day 91	11/22-23/10
Collection of Samples for Total Chromium and Iron Analyses	Day 91	11/8/10
Collection of Samples for	Day 91	11/2/10

Event	Sequence	Dates
Evaluation of Iron Status		
Necropsy for Toxicology and Histology	Day 8 Day 91	9/3/10 11/1-2/10
Draft Report Due	One draft report will be issued. The data of submission of the draft report will be determined at a later date, and will be added to this protocol by amendment.	TBD
Final Report Due	15 working days after receipt of Sponsor comments on the draft report	---

6.0 STUDY PERSONNEL:

Study Director: Charles D. Hébert, Ph.D., D.A.B.T.
Phone: (205) 581-2285
E-Mail: hebert@southernresearch.org

7.0 TEST ARTICLE & VEHICLE:

7.1 TEST ARTICLE:

Name: **Sodium dichromate dihydrate (SDD)**
Supplier: Sponsor
Lot Number(s): 05914AS
Special Handling: Handle bulk as hazardous material

Color Assignment: The color "green" has been designated to identify this test article.

Characterization: The bulk test article will be provided by the Sponsor. Test article identity, strength, quality, stability, composition, and purity, as well as methods of synthesis, fabrication, or derivation, are the responsibilities of the Sponsor. A copy of the Certificate(s) of Analysis will be included in the study file.

Stability & Storage: The test article will be supplied as bulk test article and will be stored at room temperature in glass containers and protected from light.

Disposition: Unused test article will be maintained at Southern Research until instructions on disposition are received from the Sponsor's Representative. The residual bulk test article

(with the exception of the reserve sample) will be disposed of as directed by the Sponsor's Representative.

Reserve Samples: Reserve samples from each lot of test article used on the study will be retained and stored frozen at approximately -20 °C or lower.

7.2 VEHICLE

Name: **Tap Water**
Supplier: Birmingham Water Works
Lot Number(s): Not applicable
Special Handling: None

Characterization: Not applicable

Stability & Storage: Not applicable. Tap water will be used directly from the source, and will not be stored.

Disposition: Not applicable.

Reserve Samples: Reserve samples of vehicle will be retained and stored frozen at approximately -20 °C or lower.

7.3 FORMULATIONS:

Preparation: Dose formulations of SDD will be prepared and vehicle control formulations (tap water) will be collected once during Week -2 and at 2-week (i.e., 10- to 17-day) intervals thereafter throughout the study.

Dose Formulation Analyses: Samples of each batch of SDD dose formulations from the first, third, fifth, and last mixes will be collected and shipped to a Sponsor-designated laboratory for concentration analysis. The results of these analyses will be provided to Southern Research by the Sponsor and will be included in the study records and the report. Because dose formulations of SDD in tap water are solutions, it will not be necessary to demonstrate homogeneity of the formulations used in this study. Information on the designated laboratory will be included in the study records.

Formulation Storage, Stability, and Handling: When not in use, dose formulations of SDD and vehicle formulations will be stored in sealed Nalgene (or equivalent) containers at room temperature protected from light. SDD has been shown to be stable for 42 days in dosed water formulations at a concentration of 41.8 mg/L when stored under these conditions (NTP Technical

Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). 2008. NTP TR 546). Reserve samples of each formulation will be retained and stored at approximately -70 °C.

SDD formulations in tap water have been shown to be stable under simulated animal room conditions (i.e., ambient temperature in glass bottles) for at least 7 days (NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). 2008. NTP TR 546).

Disposition: Residual formulations remaining after dose administration is complete will be disposed of as hazardous waste.

8.0 TEST SYSTEM:

Species & Strain:	Fischer Rats
Supplier:	Charles River Laboratories International, Inc.
Age on Day 1:	5-7 Weeks
Weight on Day 1:	70-110 g
Number & Sex on Study:	Females – 450

8.1 JUSTIFICATION:

Justification for Test System: The Fischer rat model was selected for this study because it is the species/strain that was used in previous studies conducted by the National Toxicology Program (NTP). The data from the current study are intended to provide information on mechanisms of action of the test article.

Justification for Number on Study: The number of animals proposed for use on this study was selected to be the minimum number needed to obtain reliable statistical results in light of limitations on the amount of blood and tissue that can be collected from a rat. Because of the small size of the target tissues, it is not feasible to collect samples of those tissues for all the necessary evaluations from the same rats. Therefore, it will be necessary to use separate cohorts of rats for collection of samples for histopathologic, biochemical, gene expression, and mutation analyses. The number of dose groups is the fewest possible consistent with the objective of the study, the scientific needs of the Sponsor, contemporary scientific standards, and applicable regulatory requirements.

Rationale for the Use of Animals for this Study: The current state of scientific knowledge does not provide acceptable alternatives, in vitro or otherwise, to the use of live animals to accomplish the purpose of this study.

Justification for Dose Levels and Route: The oral route of administration was selected for this study because this is one of the likely routes of exposure in humans. The dose levels were chosen to bracket those used in the previous NTP studies, and to provide an environmentally relevant concentration on the low end of the dose curve.

8.2 HOUSING:

Animals will be group housed (5/cage) in solid bottom cages on stainless steel racks. Irradiated hardwood bedding chips (Sani Chips®; P.J. Murphy Forest Products Corp.; Montville, NJ) will be used. Analytical reports for the bedding will be reviewed by Southern Research's veterinarian to assure that no known contaminants are present that could interfere with or affect the results of the study.

Animals will be housed in an environmentally monitored, well-ventilated room maintained at a temperature of 69–75 °F and a relative humidity of 35%-65%. Fluorescent lighting will provide illumination approximately 12 hours per day.

8.3 DIET:

Rats will be fed irradiated NTP-2000 Wafers (Zeigler Bros.; Gardners, PA) during the pre-study and study periods. Feed will be provided ad libitum. Analyses of the feed, provided by the manufacturer, will be reviewed by Southern Research's Veterinarian, or designee, to assure that no known contaminants are present that would interfere with or affect the outcome of studies.

8.4 WATER:

The water source will be the Birmingham municipal water supply. Water (either undosed for control animals or containing SDD for treated animals) will be supplied in amber glass water bottles. Teflon®-lined lids with stainless steel, double-balled sipper tubes will be used. Water bottles will be changed twice weekly on a 3-day/4-day schedule, or more frequently as needed. Samples of water from the animal facility will be periodically analyzed, and the analyses will be reviewed by Southern Research's Veterinarian, or designee, to assure that no known contaminants are present that could interfere with or affect the outcome of studies. Water bottles and sipper tubes will be labeled with color-coded zip ties to indicate the chemical and dose concentration.

8.5 ACCLIMATION:

Rats will be acclimated for a minimum of 7 days. Prior to study start, the animals will be observed for general health and acceptability for use in this study. Only animals deemed healthy will be included in this study.

8.6 ANIMAL IDENTIFICATION:

The animal identification number for each rat will consist of a letter designating the dose group, a letter designating the sex, and a unique number (e.g., UF12). The rats will be uniquely identified by tail tattoo using the numerical portion, but not the letter portion, of the identification.

9.0 EXPERIMENTAL DESIGN:

The Provantis application (Version 7; Instem Life Sciences Systems, Ltd.; Staffordshire, United Kingdom) will be used for the direct on-line capture of most in-life and pathology data. In addition, Provantis will interface with the Cobas c501 Clinical Chemistry Analyzer (Version 04-02; Roche Diagnostics; Indianapolis, IN) for capture of serum iron data. Environmental monitoring of animal rooms (i.e., temperature/humidity and light/dark cycles) will be performed using the Edstrom Watchdog System (Version 5.11; Edstrom Industries, Inc.; Waterford, WI). The remainder of the data will be collected manually or by the appropriate automated system.

9.1 RANDOMIZATION & GROUP ASSIGNMENT:

In order to obtain groups that are comparable by body weight, all rats will be assigned to their respective treatment groups using a computer-generated randomization procedure. Because of the number of rats in the study, the animals will be received in two separate shipments (cohorts), and the rats in the different cohorts will be randomized separately. The body weights required for randomization will be determined during the week prior to randomization. After randomization, rats will be assigned to one vehicle control group or to one of five treated groups as indicated below.

Group	Treatment	Conc. (mg/L)	Number of Animals							
			Toxicology and Histopathology		Biochemical Evaluations		Gene Expression Analysis		Mutation Analysis	Chromium/ Iron Analysis
			Day 8	Day 91	Day 8	Day 91	Day 8	Day 91	Day 91	Day 91
1	Water	0	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
2	SDD	0.3	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
3	SDD	4	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
4	SDD	60	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
5	SDD	170	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F
6	SDD	520	5 F	10 F	10 F	15 F	10 F	10 F	10 F	5 F

Color codes and letter designations will be assigned to the dose groups as follows:

Group	Treatment	Conc. (mg/L)	Letter Code	Color Code
1	Water	0	U	Black
2	SDD	0.3	L	Grey
3	SDD	4	I	Yellow
4	SDD	60	M	Purple
5	SDD	170	N	Blue
6	SDD	520	H	Red

9.2 DOSE PROCEDURE:

In order to accommodate necropsy and sample collections on large numbers of rats the study will be stagger-started, with Days 1 distributed as shown in Section 5.0 (Key Study Dates) of this protocol.

Rats in this study will receive the test article in their drinking water. The test article will be available ad libitum to study animals 7 days per week (including holidays) for 7 or 90 days, as shown in the table above.

In the event unanticipated, severe toxicity should be encountered in any dose group, dose groups or dosing schedules may be adjusted. The decision to exercise the option of dose reduction or schedule adjustment will be made by the Study Director or designee, in conjunction with the Sponsor's Representative if possible, based upon the nature of clinical signs that are present and severity of the condition of the animals.

9.3 FASTING REQUIREMENTS:

Fasting is not required during the course of this study.

9.4 CLINICAL OBSERVATIONS:

Daily Observations: All animals will be observed at least twice daily during the pre-study and study periods for signs of mortality and moribundity.

Detailed Observations: Each animal will be removed from its cage and examined for clinical signs of toxicity on Day 1 and weekly thereafter. Detailed clinical observations may be assessed more frequently as clinical signs warrant.

9.5 BODY WEIGHTS:

Each animal will be weighed during Week -1 for randomization, on Day 1, weekly thereafter, and prior to scheduled euthanasia. In addition, a body weight will be collected on any animal that is euthanized in extremis; body weights will not be collected for animals found dead. Body weights may be collected more frequently if deemed necessary by the Study Director.

9.6 FOOD AND WATER CONSUMPTION:

Quantitative food and water consumption will be measured by cage weekly for each cage of animals throughout the study. Values will be reported as an average consumption (grams/animal/day).

9.7 MUTATION ANALYSIS:

Samples for mutation analysis will be collected on Day 91. Ten rats/group will be euthanized using CO_2, and samples of oral epithelium and duodenal epithelium will be collected and snap frozen. Samples for mutation analysis will also be collected prior to the necropsy of any rats designated for mutation analysis that is euthanized in a moribund condition. These samples will be stored frozen at approximately -20 °C or lower until they are shipped to a Sponsor-designated laboratory for analysis. Following collection of these tissues, rats designated for mutation analysis will be discarded without further evaluation.

9.8 GENE EXPRESSION ANALYSIS:

Samples for gene expression analysis will be collected on Days 8 and 91. On each of these days, 10 rats/group will be euthanized using CO_2, and samples of oral epithelium, duodenal epithelium, and jejunal epithelium will be collected. Details on handling and storage of samples after collection will be documented in the study record. Samples for gene expression analysis will also be collected prior to the necropsy of any rat designated for gene expression analysis that is

euthanized in a moribund condition. These samples will be stored at Southern Research until they are shipped to a Sponsor-designated laboratory for analysis.

Following collection of these samples, carcasses and remaining tissues from rats designated for gene expression analysis will be discarded without further evaluation.

9.9 BIOCHEMICAL ANALYSES:

Sample Collection: Samples for biochemical analyses will be collected from 10 rats/group on Day 8 and from 15 rats/group on Day 91. On Days 8 and 91, 10 rats/group will be designated as Subgroup A; on Day 91, the remaining 5 rats/group will be designated as Subgroup B.

On Days 8 and 91, 5 rats/group in Subgroup A will be used for collection of samples for GSH/GSSG analysis and 5 rats/group in Subgroup A will be used for collection of samples for DNA-Cr adduct analysis.

Collection of Blood for GSH/GSSG Analysis (Subgroup A). For collection of blood samples for GSH/GSSG analysis, each rat will be anesthetized with ketamine/xylazine (approximately 87 mg ketamine/kg; 13.4 mg xylazine/kg) injected intraperitoneally. Should the initial dose of anesthetic fail to produce the required level of anesthesia to allow blood collection, animals may, if necessary, be administered another half dose of ketamine (approximately 43-44 mg/kg). Should this additional dose fail to produce the required level of anesthesia, the rats will be anesthetized with CO_2/O_2 administered by inhalation. Blood samples will be collected from the retro-orbital plexus into tubes containing heparin as anticoagulant (Subgroup A). Samples will be gently mixed by inversion and placed on ice. Within 15 minutes of collection, samples will be centrifuged for approximately 5 minutes under refrigerated conditions for separation of plasma. Plasma will be collected, and mixed in a 1:1 ratio with 2X Redox Quenching Buffer (RQB), to yield final concentrations of 20 mM HCl, 5 mM diethylenetriamine pentaacetic acid, and 1 mM 1,10-phenanthroline. The 2X RQB will also contain 5% ultrapure grade trichloroacetic acid. Samples will then be mixed by gentle inversion and snap frozen until they are shipped to a Sponsor-designated laboratory for analysis.

Collection of Blood for Cytokine Analysis (Subgroup B). For collection of blood samples for cytokine analysis, each rat will be anesthetized with CO_2/O_2, and blood samples will be collected from the retro-orbital plexus into serum separator tubes containing no anticoagulant (Subgroup B). The contents of the Subgroup B tubes will be centrifuged to separate serum. Prior to euthanasia, the orbital route may be supplemented by puncture of the vena cava if necessary. Blood samples

for biochemical analysis will also be collected prior to the necropsy of any rat designated for biochemical analysis that is euthanized in a moribund condition.

Collection of Tissues for GSH/GSSG and DNA-Cr Adduct Analysis (Subgroup A). Immediately following blood collection, each rat will be euthanized using CO_2. Samples of oral epithelium, duodenal epithelium, and jejunal epithelium will be collected from animals in Subgroup A. Tissues for GSH/GSSG analysis will be immediately placed into tubes containing 0.5 mL 2X RQB on ice. The tissues will be allowed to sit in RQB on ice for approximately 10-15 minutes to allow penetration of the buffer into the tissues, then the tubes will be snap frozen in liquid nitrogen. Tissues for DNA-Cr adduct analysis will be placed into tubes and snap frozen without buffer.

Collection of Tissues for 8-OHdG, Cytokine, and 8-Isoprostane Analysis (Subgroup B). A sample of oral mucosa and underlying muscle and an intact segment from the cranial end of the duodenum will be collected from each animal in Subgroup B. Following collection of these tissues, rats designated for biochemical analysis will be discarded without further evaluation.

Handling and Shipping of Samples. Plasma and tissue samples from animals in Subgroup A will be stored frozen (at or below -70 °C) until they are shipped to Sponsor-designated laboratories for analysis. Serum samples from animals in Subgroup B will be divided into two aliquots, and the oral cavity and duodenum samples will be weighed and split into two pieces longitudinally (if possible). Serum and tissue samples will be snap-frozen upon collection, and will be stored frozen (at or below -70 °C) until they are used for analysis.

Biochemical Analysis, GSH/GSSG Ratio: One plasma sample, one sample of oral epithelium, one sample of duodenal epithelium, and one sample of jejunal epithelium from each of 5 animals/group (Day 8 and Day 91 collections) in Subgroup A will be shipped to a Sponsor-designated laboratory for analysis of GSH and GSSG, and subsequent calculation of GSH/GSSG ratios. The recipient laboratory will be documented in the study records.

Biochemical Analysis, DNA-Cr Adducts: One sample of oral epithelium, one sample of duodenal epithelium, and one sample of jejunal epithelium from each of 5 animals/group (Day 8 and Day 91 collections) in Subgroup A will be shipped to a Sponsor-designated laboratory for analysis. The recipient laboratory will be documented in the study records.

Biochemical Analysis, 8-OHdG: One sample of oral cavity and one sample of duodenum from each of 5 animals/group in Subgroup B will be analyzed for 8-OHdG.

Biochemical Analysis, Cytokines: One serum sample, one sample of oral cavity, and one sample of duodenum from each of 5 animals/group in Subgroup B will be analyzed for IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, TNF-α, KC/GRO, MCP-1, G-CSF, GM-CSF, IP-10, MIP-1α, RANTES, and IFN-γ.

Biochemical Analysis, 8-Isoprostane: One sample of oral cavity and one sample of duodenum from each of 5 animals/group in Subgroup B will be analyzed for 8-isoprostane.

9.10 TOTAL CHROMIUM AND IRON ANALYSIS:

Samples for evaluation of total chromium and iron content will be collected on Day 91. Five rats/group will be anesthetized with CO_2/O_2, and blood will be collected from the retro-orbital plexus into tubes containing lithium heparin as anticoagulant. Samples will be gently mixed by inversion and placed on ice until they are centrifuged to separate plasma. The erythrocyte layer and the plasma layer will be collected separately, snap-frozen, and stored at approximately -20 °C.

Following blood collection, the tissues in the list below will be collected from each animal, weighed, and snap frozen.

Following blood collection, rats will be euthanized using CO_2, and organs/tissues will be collected intact, snap-frozen, and stored at approximately -20 °C. Prior to freezing, the length of the intestinal segments (duodenum, ileum, and jejunum) will be recorded. The following organs/tissues will be collected:

Bone (femur)
Glandular stomach (flushed of contents)
Kidney
Liver
Oral mucosa
Small intestine, duodenum (flushed of contents)
Small intestine, jejunum (flushed of contents)
Small intestine, ileum (flushed of contents)
Spleen

Plasma, red blood cells, and tissue samples will be stored frozen at approximately -20 °C until they are shipped to a Sponsor-designated laboratory for analysis.

Samples for total chromium and iron analysis will also be collected prior to the necropsy of any rat designated for these groups that is euthanized in a moribund condition. Following collection of these tissues, rats designated for these groups will be discarded without further evaluation.

9.11 EVALUATION OF IRON STATUS:

Prior to euthanasia, half of the rats designated for macroscopic and microscopic pathology evaluation (i.e., those in the Toxicology and Histopathology groups) will also be used for collection of samples for evaluation of iron status.

Sample Collection: On Day 91, five rats per group will be anesthetized using CO_2/O_2, and blood samples (~1.0 mL) will be collected from the retro-orbital plexus into tubes containing no anticoagulant. The contents of the tubes will be centrifuged to separate serum. Serum samples will be aliquotted into four aliquots, one of which will be used for measurement of serum iron on the day of collection. The remaining three aliquots will be snap frozen and stored at approximately -20 °C until used for ELISA analysis.

After collection of serum samples, rats will be euthanized for gross and microscopic pathology as described below. One bone marrow smear will be prepared from each rat.

Measurement of Serum Iron: One serum aliquot will be used for measurement of serum iron levels using the Cobas c501 Clinical Chemistry Analyzer (Version 04-02; Roche Diagnostics; Indianapolis, IN).

Evaluation of Bone Marrow Smears: Bone marrow smears will be stained using a stain that will allow visualization of iron (specific stain to be documented in the study records), and will be evaluated by a board-certified clinical pathologist to estimate iron content.

ELISA Analysis of Serum Samples: Two frozen serum samples will be analyzed for serum ferritin and serum transferrin using commercial ELISA kits. The third will be retained for possible late analysis of serum hepcidin. If required, analysis of serum hepcidin will incur additional cost.

9.12 MACROSCOPIC AND MICROSCOPIC PATHOLOGY:

Macroscopic Pathology: Rats designated for macroscopic and microscopic pathology evaluation (i.e., those in the Toxicology and Histopathology groups) will be euthanized by CO_2 asphyxiation on Day 8 (5 rats/group) and Day 91 (10 rats/group). In the event that some rats die on study, the distribution of animals necropsied on the two necropsy days will be determined by the Study Director with concurrence from the Sponsor.

Rats in the groups designated for pathologic examination that are euthanized at scheduled necropsy, and any that are found dead or euthanized in extremis will be

subjected to a complete gross necropsy examination. The postmortem examination of each rat will include, but not be limited to, examination of the external surfaces of the body, all orifices of the body, and the cranial, thoracic, abdominal, and pelvic cavities and their contents.

The oral cavity, duodenum, jejunum, and any gross lesions will be collected from each rat and saved in 10% neutral buffered formalin for histopathologic evaluation. The animal identification will be collected, fixed in 10% neutral buffered formalin, and retained with its tissues collected during necropsy.

In addition, for animals in histopathology groups 1, 2, 4, and 6 on Days 8 and 91, the esophagus, stomach (forestomach and glandular), liver, and mesenteric lymph nodes will be collected. Each tissue will be divided into two samples (tissues split longitudinally where possible) and saved for possible future evaluation. One piece of each tissue from each animal will be fixed in 10% neutral buffered formalin, and the other will be snap frozen and stored at -20 °C or lower.

Organ Weights: Organ weights will not be required.

Histology: The oral cavity, duodenum, jejunum, and any gross lesions from each rat in the Toxicology and Histopathology groups will be processed to slides. The fixed tissues will be trimmed, processed, and microtomed (approximately 5-µm sections), and the tissue sections will be mounted on glass slides; ten slides of each tissue will be prepared. One of the ten slides from each tissue of each animal will be stained with hematoxylin and eosin, and coverslipped. The remaining nine samples will be retained for possible future use. Special stains may be applied at the discretion of the pathologist when necessary to establish a diagnosis.

Microscopic Observations: All slides will be submitted to a veterinary pathologist for evaluation and diagnosis. For tissues from animals in the Toxicology and Histopathology groups, findings will be diagnosed and categorized using standardized nomenclature with lesions ranked for severity for comparison among groups.

9.13 EARLY TERMINATION:

In the event unanticipated, severe toxicity should be encountered in any dose group, animals or entire dose groups may be terminated early. The decision to exercise the option of early termination will be made by the Study Director or designee, in conjunction with the Sponsor's Representative if possible, based upon the nature of clinical signs that are present and severity of the condition of the animals.

10.0 STATISTICAL ANALYSIS:

Group means and standard deviations will be calculated when appropriate for body weights, food and water consumption data, biochemical assay data, and any other data deemed appropriate by the Study Director. Evaluation of data for the differences between groups (body weight data, food and water consumption data, and any other data deemed appropriate by the Study Director) will utilize ANOVA and Dunnett's Test for multiple comparisons. Additional statistical tests may be applied if deemed necessary.

Statistical analysis will be performed using the Provantis automated data collection system (Instem; Staffordshire, UK) at Southern Research, unless otherwise deemed necessary by the Study Director. If a consultant statistician is used for selected analyses, the name, credentials, contact information, and specific data evaluated will be documented in the study record. In all cases, the lower limit for statistical significance will be defined as $p \leq 0.05$. The following inter-group comparisons will be made:

- Group 1 to Groups 2, 3, 4, 5, and 6

11.0 RECORDS:

All raw data pertaining to the conduct of this study, and all samples/specimens generated in this study and either analyzed at Southern Research or retained for storage, will be stored at Southern Research for up to 1 year after the issuance of the draft report. After 1 year, or at any time prior to the completion of that year if the Sponsor's Monitor so directs, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor's designated archival facility. The Sponsor must approve the final disposition of all raw data and samples/specimens generated in this study. The original final report will be retained in the central Archives at Southern Research.

12.0 REPORT:

A single draft report will be prepared and issued to the Sponsor; the date of submission of this report will be determined at a later time, and will be added to this protocol by amendment. The final report will be issued within 15 working days after receipt of the Sponsor's review comments on the draft report. If Sponsor comments on the draft report are not received at Southern Research within 60 working days after submission of the report, the draft report will be issued as final.

The determination as to whether or not the results from analyses performed at sites other than Southern Research will be reported in the Southern Research study report will be made by the Sponsor at a later date and documented in the study records.

13.0 REGULATORY REFERENCES:

This study will be conducted in accordance with the protocol, the Standard Operating Procedures (SOPs) of Southern Research, and the applicable regulatory requirements, as addressed below.

13.1 PROTOCOL AMENDMENTS AND DEVIATIONS:

Amendments: All changes in or revisions of the approved protocol and the reasons for these changes will be documented in amendments, which will be signed and dated by the Study Director and the Sponsor's Representative. Amendments will be maintained with the protocol. Written approval (a fax signature or electronic communication, such as email) for changes in the protocol may be granted by the Sponsor's Representative, but a written amendment will follow.

Deviations: All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the SOPs of Southern Research and/or the protocol, and any deviations from protocol or SOPs will be documented.

13.2 REGULATORY COMPLIANCE:

Good Laboratory Practices: This nonclinical laboratory study will not be conducted in compliance with the Good Laboratory Practice (GLP) Regulations of the U.S. Food and Drug Administration (FDA) (21 CFR Part 58) or the U.S. Environmental Protection Agency (40 CFR Part 792).

Quality Assurance Review: The study described in this protocol will not be subjected to Quality Assurance evaluations of the laboratory processes at Southern Research, data, and the final report.

13.3 FACILITIES MANAGEMENT AND ANIMAL HUSBANDRY:

General procedures for animal care and housing will be in compliance with the SOPs of Southern Research, the *Guide for the Care and Use of Laboratory Animals,* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council; National Academy Press; Washington, DC), and the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99-198). Southern Research is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

13.4 ANIMAL WELFARE ACT COMPLIANCE:

By signing this protocol, the Sponsor signifies that there are no generally accepted alternatives to the use of animals, and that the study described by this protocol does not unnecessarily duplicate previously conducted or reported experiments.

Procedures used in this protocol are designed to conform to accepted practices and to minimize or avoid causing pain, distress, or discomfort in the animals. In those circumstances in which required study procedures are likely to cause more than momentary or slight pain or distress, the animals will receive appropriate analgesics or anesthetics unless the withholding of these agents has been justified in writing by the Study Director and/or the Sponsor and approved by the Institutional Animal Care and Use Committee at Southern Research.

14.0 PROTOCOL APPROVALS:

This protocol has been reviewed and approved.

Study Director: Charles D. Hébert 7-21-10

Charles D. Hébert, Ph.D., D.A.B.T. Date

Sponsor's Representative: 8/5/10

Mark A. Harris, Ph.D. Date

PROTOCOL AMENDMENT: 13026.01.02A$_1$

Protocol 13026.01.02 is amended as follows:

1. Page 8 — Section 8.6 (Animal Identification): The original protocol stated that the animal identification number for each animals will consist of a letter designating the dose group, a letter designating the sex, and a unique number.

   In order to better accommodate data collection capabilities of the Provantis system, the protocol is amended to remove the requirement for a letter designation for each dose group. The revised Section 8.6 is presented below in its entirety. Changes are indicated in **bold underlined** font.

   8.6 ANIMAL IDENTIFICATION:

   The animal identification number for each rat will consist of a **number** designating the dose group, a letter designating the sex, and a unique number (e.g., **1**F12). The rats will be uniquely identified by tail tattoo using the **unique** numerical portion, but not the **dose group or sex** letter portion, of the identification.

2. Page 9 — Section 9.1 (Randomization and Group Assignment): The original protocol included a table that showed a letter designation for each dose group. The letter designation is being replaced by a dose group number. The revised table and associated text are presented below. Changes are indicated in **bold underlined** font.

   Color codes and **number** designations will be assigned to the dose groups as follows:

Group	Treatment	Conc. (mg/L)	Color Code
1	Water	0	Black
2	SDD	0.3	Grey
3	SDD	4	Yellow
4	SDD	60	Purple
5	SDD	170	Blue
6	SDD	520	Red

Effective date of these items: July 23, 2010

Approval Signatures:

Study Director: Charles D. Hébert — 7-23-10
Charles D. Hébert, Ph.D., D.A.B.T. — Date

Sponsor's Representative: Mark A. Harris — 8/5/10
Mark A. Harris, Ph.D. — Date

PROTOCOL AMENDMENT: 13026.01.02A$_2$

August 2, 2010
Page 1 of 1

Protocol 13026.01.02 is amended as follows:

1. Page 13 Section 9.9 (Biochemical Analyses; Biochemical Analysis, Cytokines): The original protocol stated that samples would be analyzed for IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12p70, IL-13, IL-15, IL-17, TNF-α, KC/GRO, MCP-1, G-CSF, GM-CSF, IP-10, MIP-1α, RANTES, and IFN-γ. These were the same cytokines analyzed in a previous study. However, the assay kit to be used for the rats contains a slightly different panel of cytokines. The revised paragraph is presented below in its entirety. Changes are indicated in **bold underlined** font.

Biochemical Analysis, Cytokines: One serum sample, one sample of oral cavity, and one sample of duodenum from each of 5 animals/group in Subgroup B will be analyzed for IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-13, IL-17, **IL-18,** TNF-α, KC/GRO, MCP-1, G-CSF, GM-CSF, IP-10, MIP-1α, RANTES, **eotaxin, leptin, VEGF,** and IFN-γ.

Effective date of these items: August 2, 2010

Approval Signatures:

Study Director: Charles D. Hébert 8-2-10
Charles D. Hébert, Ph.D., D.A.B.T. Date

Sponsor's Representative: 8-3-10
Mark A. Harris, Ph.D. Date

PROTOCOL AMENDMENT: 13026.01.02A$_3$

Protocol 13026.01.02 is amended as follows:

1. Page 3 Section 5.0 (Key Study Dates): The original protocol stated that the submission date of the draft study report would be added to the protocol by amendment. The relevant line of the Key Study Dates table is amended as shown below. Changes are shown in bold font.

Event	Sequence	Dates
Draft Report Due	**One draft report will be issued approximately 12 weeks after removal of the final animal in the study.**	**2/16/11**

2. Page 15 Section 12.0 (Report): The original protocol stated that the date of submission of the draft report would be determined at a later time, and would be added to the protocol by amendment. The first sentence of this section is amended to read.

"A single draft report will be prepared and issued to the Sponsor approximately **12 weeks after removal of the final animal in the study**."

Effective date of these items: October 5, 2010

Approval Signatures:

Study Director: Charles D. Hébert 10-5-10
Charles D. Hébert, Ph.D., D.A.B.T. Date

Sponsor's Representative: 10/12/10
Mark A. Harris, Ph.D. Date

PROTOCOL AMENDMENT: 13026.01.02A$_4$

Protocol 13026.01.02 is amended as described below. Changes are shown in **bold font and underlined**.

1. Page 3

Section 5.0 (Key Study Dates): Because of a problem with processing of bone marrow slides collected for evaluation of iron status on November 2, 2010, it is necessary to repeat the collection of all iron status evaluation samples. These samples will be collected from the animals necropsied on November 22, 2010 for mutation analysis, and will be in addition to the mutation analysis samples. The relevant line of the Key Study Dates table is amended as shown below.

Event	Sequence	Dates
Collection of Samples for Evaluation of Iron Status	Day 91	11/2/10 **11/22/10**

2. Page 10

Section 9.7 (Mutation Analysis): Because of the need to collect additional samples for evaluation of iron status, this section is amended to note that the additional samples will be collected from five animals per group in the cohort used for mutation analysis. These iron status samples will be in addition to the samples collected for mutation analysis. The procedures necessary for collection of the iron status samples (e.g., anesthesia) will be in addition to those already described for mutation analysis. The revised first sentence of this section now reads:

"Samples for mutation analysis will be collected on Day 91**; in addition, five rats/group from the animals used for mutation analysis will be used for collection of samples for evaluation of iron status as described in Section 9.11**.

3. Page 14

Section 9.11 (Evaluation of Iron Status): Because of the need to collect additional samples for evaluation of iron status, this section is amended to note that the additional samples will be collected from five animals per group in the cohort used for mutation analysis, and to describe handling of samples. The first paragraph of this section now reads:

"Prior to euthanasia, half of the rats designated for macroscopic and microscopic pathology evaluation (i.e., those in the Toxicology and Histopathology groups)**, and half of the rats designated for mutation analysis** will also be used for collection of samples for evaluation of iron status."

The third paragraph of this section now reads:

"After collection of serum samples, rats will be euthanized for gross and microscopic pathology as described below**, or for mutation analysis as**

described in Section 9.7. One bone marrow smear will be prepared from each rat **in the Toxicology and Histopathology groups, and three bone marrow smears will be prepared from each rat in the mutation analysis groups. In addition, one femur from each rat in the mutation analysis groups will be collected, fixed in formalin, and retained for possible future use.**"

The fifth paragraph of this section now reads:

"**Evaluation of Bone Marrow Smears and Formalin-Fixed Bone:** Bone marrow smears **(and, if deemed necessary by the Study Director, formalin-fixed bone)** will be stained using a stain that will allow visualization of iron (specific stain to be documented in the study records), and will be evaluated by a board-certified clinical pathologist to estimate iron content."

The sixth paragraph has been revised to (1) add a description of how serum samples for ELISA analysis that are collected from the rats used for mutation analysis will be analyzed, (2) note that serum samples for ELISA analysis that are collected from the rats used for gross and microscopic analysis will be retained for possible future use, and (3) correct a minor typographical error in the original protocol. This section now reads:

"**ELISA Analysis of Serum Samples:** Two frozen serum samples **collected from the rats used for mutation analysis** will be analyzed for serum ferritin and serum transferrin using commercial ELISA kits. The third will be retained for possible future later analysis of serum hepcidin. If required, the analysis of serum hepcidin will incur additional cost. **The frozen serum samples collected from the rats used for gross and microscopic pathology will be retained for possible future use.**

4. Page 16 Section 12.0 (Report): Because of the problem with processing of bone marrow slides collected from animals in the Toxicology and Histopathology groups, these slides were not evaluable, and no data are available. However, serum iron analyses for these animals had already been performed. This section is revised to describe handling of this serum iron data. A new third paragraph in this section reads:

"**The decision whether to include in the final report the data from serum iron samples collected from animals in the Toxicology and Histopathology groups will be made by the Sponsor and documented in the study records.**"

Effective date of these items: November 19, 2010

Approval Signatures:

Study Director: Charles D. Hébert 11-19-10
Charles D. Hébert, Ph.D., D.A.B.T. Date

Sponsor's Representative: 11-20-10
Mark A. Harris, Ph.D. Date

Appendix B

Sodium Dichromate Dihydrate Certificate of Analysis

Certificate of Analysis

SIGMA-ALDRICH

Product Name	Sodium dichromate dihydrate, 99.995% trace metals basis
Product Number	483060
Product Brand	ALDRICH
CAS Number	7789-12-0
Molecular Formula	$Na_2Cr_2O_7 \cdot 2H_2O$
Molecular Weight	298.00

TEST	**LOT 05914AS RESULTS**
APPEARANCE	ORANGE CRYSTALS
INFRARED SPECTRUM	CONFORMS TO STRUCTURE.
TITRATION	34.8% CR (WITH SODIUM THIOSULFATE)
ATOMIC ABSORPTION	K 50 PPM
TRACE ANALYSIS, ICP	AG 1 PPM
	CA 1 PPM
	FE 0.8 PPM
ICP ASSAY	CONFIRM SODIUM AND CHROMIUM COMPONENTS.
QUALITY CONTROL	FEBRUARY 1998
ACCEPTANCE DATE	

Barbara Rajzer, Supervisor
Quality Control
Milwaukee, Wisconsin USA

Appendix C

Individual Clinical Observations

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
1	f	1	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		2	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		3	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		4	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		5	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		6	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		7	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		8	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		9	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		10	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		11	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		12	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	.	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	.	.	R	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		13	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
1	f	14	No Abnormalities Detected		X	X	X	X	X	.	.	.	X	.	.	X	X	X
			Discharge	Right eye	.	.	.	.	.	R	R	R	.	R	.	.	.	.
			Hyperexcitable		.	.	.	.	.	.	.	.	.	.	X	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		15	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		16	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		17	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		18	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		19	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		20	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		21	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		22	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		23	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		24	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		25	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		26	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
1	f	27	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		28	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		29	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		30	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		31	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		32	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		33	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		34	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		35	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		36	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		37	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		38	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
1	f	39	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		40	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		41	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		42	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		43	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		44	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		45	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		46	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		47	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		48	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		49	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		50	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		51	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
1	f	52	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		53	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	X	X	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	R	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		54	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		55	No Abnormalities Detected		X	X	X	X	X	X	.	X	X	X	X	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	R	.	.	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		56	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		57	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		58	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		59	No Abnormalities Detected		X	X	X	X	X	X	.	.	.	.	.	.	.	.
			Alopecia	Both forelimbs	.	.	.	.	.	.	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		60	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		61	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		62	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		63	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
1	f	64	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		65	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		66	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		67	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		68	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		69	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		70	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		71	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		72	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		73	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		74	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		75	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
2	f	76	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		77	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		78	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		79	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		80	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		81	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		82	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		83	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		84	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		85	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		86	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		87	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		88	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		89	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
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90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
2	f	90	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		91	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		92	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		93	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		94	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		95	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		96	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		97	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	R	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		98	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		99	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		100	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		101	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		102	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
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90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
2	f	103	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		104	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		105	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		106	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		107	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		108	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		109	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		110	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		111	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		112	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		113	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		114	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		115	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
2	f	116	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	X
			Alopecia	Both forefeet	.	.	.	.	.	.	.	.	.	.	.	X	X	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		117	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		118	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		119	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		120	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		121	No Abnormalities Detected		X	X	X	X	X	X	X	.	.	X	X	X	X	X
			Alopecia	Both forelimbs	.	.	.	.	.	.	.	X	X	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		122	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		123	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		124	No Abnormalities Detected		X	X	X	X	X	X	X	.	.	.	.	.	X	X
			Alopecia	Both forelimbs	.	.	.	.	.	.	.	X	X	X	X	X	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		125	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		126	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	X	X
			Discharge	Both eyes	.	.	.	.	.	.	.	.	.	R	R	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		127	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
2	f	128	No Abnormalities Detected		X	X	X	X	X	X	.	X	X	.	X	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	R	.	.	R	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		129	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		130	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		131	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		132	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		133	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		134	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		135	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		136	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		137	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		138	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		139	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
2	f	140	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		141	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		142	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		143	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		144	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		145	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		146	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		147	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		148	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		149	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		150	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	151	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		152	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		153	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		154	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		155	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		156	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		157	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		158	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		159	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		160	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		161	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Piloerection		.	.	.	.	.	.	.	.	.	.	.	.	.	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		162	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Piloerection		.	.	.	.	.	.	.	.	.	.	.	.	.	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
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90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	163	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Discharge	Both eyes	.	.	.	.	.	.	.	.	.	.	.	.	.	R
			Piloerection		.	.	.	.	.	.	.	.	.	.	.	.	.	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		164	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Piloerection		.	.	.	.	.	.	.	.	.	.	.	.	.	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		165	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Piloerection		.	.	.	.	.	.	.	.	.	.	.	.	.	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		166	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		167	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		168	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		169	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	.	.	R	R	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		170	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		171	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		172	No Abnormalities Detected		X	X	X	X	X	.	.	.	.	.	X	X	X	X
			Alopecia	Face	.	.	.	.	.	X	X	X	X	X	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
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90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	173	No Abnormalities Detected		X	X	X	X	X	.	.	.	.	.	X	X	X	X
			Alopecia	Face	.	.	.	.	.	X	X	X	X	X	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		174	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		175	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		176	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		177	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		178	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		179	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		180	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		181	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		182	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		183	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		184	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		185	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	186	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		187	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		188	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		189	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		190	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		191	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	.	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		192	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		193	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	.	R	R	R
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		194	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		195	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	196	No Abnormalities Detected		X	X	X	X	X	X	X	X	.	X	X	X	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	R	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		197	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		198	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		199	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		200	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		201	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		202	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		203	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	X	X	X	X
			Alopecia	Face	.	.	.	.	.	.	.	.	.	X	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		204	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		205	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Discharge	Both eyes	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		206	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Both forelimbs	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	207	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Alopecia	Both forelimbs	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		208	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		209	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Alopecia	Both forelimbs	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		210	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		211	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		212	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		213	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		214	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		215	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		216	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		217	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		218	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		219	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
3	f	220	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		221	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		222	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		223	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		224	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		225	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
4	f	226	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		227	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		228	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		229	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		230	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		231	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		232	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		233	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		234	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		235	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		236	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		237	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		238	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	.	X
			Discharge	Nose	.	.	.	.	.	.	.	.	.	.	.	.	C	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
4	f	239	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		240	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		241	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		242	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		243	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	X	X	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	R	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		244	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		245	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		246	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		247	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		248	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		249	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		250	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		251	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
4	f	252	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		253	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		254	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	X	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	.	R	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		255	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		256	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		257	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		258	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		259	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		260	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		261	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		262	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		263	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		264	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
4	f	265	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		266	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		267	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		268	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		269	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		270	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		271	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		272	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		273	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		274	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		275	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		276	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		277	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
4	f	278	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		279	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		280	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		281	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		282	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		283	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		284	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		285	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		286	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		287	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		288	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		289	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		290	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		291	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
4	f	292	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		293	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		294	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		295	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		296	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		297	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		298	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		299	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		300	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
5	f	301	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		302	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		303	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		304	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		305	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		306	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		307	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		308	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		309	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		310	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		311	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		312	No Abnormalities Detected		X	X	X	X	X	.	.	.	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Discharge	Both eyes	.	.	.	.	.	R	R	R	.	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
5	f	313	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		314	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		315	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		316	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		317	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		318	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		319	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		320	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		321	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		322	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
5	f	323	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		324	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		325	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	.	.	.
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	B	B	B	B
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		326	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		327	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		328	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		329	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		330	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		331	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		332	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		333	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	.	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	R	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		334	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		335	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
5	f	336	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		337	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		338	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	.	.	R	R
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		339	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		340	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		341	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		342	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		343	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		344	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		345	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
5	f	346	No Abnormalities Detected		X	X	X	X	.	.	.	X	X	X	X	.	X	X
			Alopecia	Face	.	.	.	.	X	X	X	.	.	.	.	.	.	.
			Discharge	Both eyes	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		347	No Abnormalities Detected		X	X	X	X	.	.	.	.	.	.	.	.	X	X
			Alopecia	Face	.	.	.	.	X	X	X	X	X	X	X	X	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		348	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		349	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		350	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		351	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Alopecia	Face	.	.	X	X	X	X	X	X	X	X	X	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	R	R	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		352	No Abnormalities Detected		X	X	X	X	X	X	X	.	.	.	X	X	X	X
			Alopecia	Face	.	.	.	.	.	.	.	X	X	X	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		353	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	.	.	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		354	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
5	f	355	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	.	X	.	X	X
			Alopecia	Face	.	.	.	.	.	.	.	.	.	X	.	.	.	.
			Discharge	Both eyes	.	.	.	.	.	.	.	.	.	.	.	C	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		356	No Abnormalities Detected		X	X	X	X	X	.	.	X	X	X	X	X	X	X
			Discharge	Left eye	.	.	.	.	.	R	R	.	.	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		357	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		358	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		359	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		360	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		361	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		362	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		363	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		364	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		365	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		366	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1 5	1 8	2 2	2 9	3 6	4 3	5 0	5 7	6 4	7 1	7 8	8 5	9 1
5	f	367	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		368	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		369	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		370	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		371	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		372	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		373	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		374	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.
		375	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
6	f	376	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		377	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		378	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		379	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		380	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		381	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		382	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		383	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		384	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		385	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		386	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		387	No Abnormalities Detected		X	X	X	X	X	X	X	X	.	.	X	X	.	.
			Discharge	Both eyes	.	.	.	.	.	.	.	.	.	R	.	.	R	R
			Discharge	Right eye	.	.	.	.	.	.	.	.	R	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
6	f	388	No Abnormalities Detected		X	X	X	X	X	X	X	.	.	.	X	X	.	.
			Discharge	Both eyes	.	.	.	.	.	.	.	R	.	R	.	.	.	.
			Discharge	Left eye	.	.	.	.	.	.	.	.	R	.	.	.	R	R
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		389	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	.	X
			Discharge	Right eye	.	.	.	.	.	.	.	.	.	.	.	.	R	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		390	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		391	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		392	No Abnormalities Detected		X	X	X	X	X	X	.	X	X	X	X	X	X	X
			Discharge	Right eye	.	.	.	.	.	.	R	.	.	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		393	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	.
			Discoloration	Vulva	.	.	.	.	.	.	.	.	.	.	.	.	.	B
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		394	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		395	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		396	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		397	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		398	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
6	f	399	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		400	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		401	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		402	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		403	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		404	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		405	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		406	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		407	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		408	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		409	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		410	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		411	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		412	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
6	f	413	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		414	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		415	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		416	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		417	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		418	No Abnormalities Detected		X	X	X	X	X	.	.	X	X	X	X	X	.	.
			Alopecia	Face	.	.	.	.	.	.	.	.	.	.	.	.	X	X
			Discharge	Both eyes	.	.	.	.	.	K	K	.	.	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		419	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		420	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		421	No Abnormalities Detected		X	X	X	X	X	X	X	X	.	X	X	.	.	.
			Discharge	Left eye	.	.	.	.	.	.	.	.	R	.	.	R	R	R
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		422	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		423	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		424	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
6	f	425	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		426	No Abnormalities Detected		X	X	X	X	X	X	.	X	X	X	X	X	X	X
			Discharge	Left eye	.	.	.	.	.	.	R	.	.	.	.	.	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		427	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		428	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		429	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	.	X	X
			Discharge	Left eye	.	.	.	.	.	.	.	.	.	.	.	R	.	.
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		430	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		431	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		432	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		433	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		434	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		435	No Abnormalities Detected		X	X	X	X	X	X	X	X	X	X	X	X	X	X
			Scheduled euthanasia		.	.	.	.	.	.	.	.	.	.	.	.	.	X
		436	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		437	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Clinical Observations

Day numbers relative to Start Date

Group	Sex	Animal	Clinical Sign	Site	1	8	15	22	29	36	43	50	57	64	71	78	85	91
6	f	438	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		439	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		440	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		441	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		442	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		443	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		444	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		445	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		446	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		447	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		448	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		449	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.
		450	No Abnormalities Detected		X	X	.	.	.	.	.	.	.	.	.	.	.	.
			Scheduled euthanasia		.	X	.	.	.	.	.	.	.	.	.	.	.	.

Severity Codes: X = Present; C = Clear; R = Red; B = Brown; K = Black

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Appendix D

Individual Body Weights

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
1	f	1	88.9	102.9	120.4	.	.	.	.	.
		2	79.9	94.3	112.5	.	.	.	.	.
		3	71.6	87.7	108.0	.	.	.	.	.
		4	73.9	86.9	109.3	.	.	.	.	.
		5	83.7	96.3	111.7	.	.	.	.	.
		6	82.5	99.8	118.8	.	.	.	.	.
		7	79.0	91.3	111.2	.	.	.	.	.
		8	68.7	85.1	106.8	.	.	.	.	.
		9	75.2	90.6	109.0	.	.	.	.	.
		10	84.9	103.6	124.1	.	.	.	.	.
		11	85.3	104.9	117.6	122.6	139.7	142.2	146.3	152.5
		12	82.1	105.3	121.1	125.1	144.5	143.0	151.9	156.6
		13	72.6	96.5	116.6	126.8	146.7	150.1	163.9	170.1
		14	74.6	90.9	105.2	112.0	125.0	130.5	137.7	137.3
		15	73.6	94.8	108.9	118.4	136.5	140.7	149.0	153.8
		16	81.2	101.6	101.2	121.2	135.4	138.8	148.0	151.3
		17	83.9	102.8	101.6	122.6	131.8	135.8	143.6	145.3
		18	89.7	106.9	108.6	131.0	140.1	146.1	153.6	158.1
		19	90.7	112.0	113.0	132.9	144.5	151.0	156.4	163.0
		20	84.4	108.7	110.4	131.4	142.2	145.8	151.9	157.2
		21	81.8	103.5	108.9	107.4	136.7	147.1	152.6	159.8
		22	86.3	107.3	109.9	108.3	134.7	145.3	152.8	156.1
		23	67.7	91.7	97.3	94.8	120.6	130.1	140.0	143.5
		24	68.2	93.2	101.2	103.0	131.8	143.1	146.4	152.1
		25	77.0	98.4	107.8	106.0	134.9	144.8	149.1	155.3
		26	69.7	100.7	107.0	110.6	142.3	150.9	156.3	161.1
		27	83.3	103.2	106.7	104.7	134.1	143.7	145.7	150.3
		28	76.8	97.3	102.2	103.1	128.4	139.4	143.1	149.8
		29	78.8	103.5	110.2	109.5	138.5	148.3	149.9	154.8

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
1	f	30	68.8	93.2	101.6	102.1	128.8	137.9	141.3	149.1
		31	70.2	98.2	103.3	124.0	137.2	142.7	151.7	153.4
		32	73.1	102.1	108.2	126.7	138.5	147.7	154.2	153.4
		33	81.1	104.5	109.6	124.0	138.4	146.7	152.8	151.4
		34	80.6	106.3	111.8	127.9	141.4	146.0	150.6	149.4
		35	80.2	104.5	105.7	118.6	130.6	135.3	142.8	143.5
		36	78.3	109.7	113.1	131.7	142.9	148.9	152.5	159.7
		37	82.8	108.2	111.9	127.3	137.2	144.6	147.7	154.0
		38	88.5	116.4	115.7	132.9	142.4	147.4	150.1	153.7
		39	86.0	115.8	115.5	130.1	139.8	144.5	149.7	154.4
		40	90.1	118.4	120.6	138.8	150.7	154.8	159.5	165.0
		41	85.5	115.5	131.3	136.4	147.3	154.9	159.9	168.3
		42	70.9	104.6	118.2	129.9	139.6	148.3	151.7	156.0
		43	87.3	116.9	127.5	137.9	145.5	153.7	154.5	162.3
		44	76.0	104.6	117.9	129.2	138.2	145.3	148.1	152.0
		45	89.1	113.4	123.0	130.9	138.5	144.5	144.8	151.9
		46	79.9	110.1	110.7	119.6	136.2	141.9	145.0	152.1
		47	90.5	119.6	118.3	133.8	146.1	150.1	155.0	159.6
		48	85.9	115.0	111.8	125.8	139.2	144.2	149.8	150.4
		49	77.5	111.8	108.6	125.8	137.1	145.5	151.5	152.8
		50	78.0	110.4	109.7	127.0	139.0	144.4	151.3	153.3
		51	87.7	103.2	118.1	128.7	137.1	146.5	151.3	155.2
		52	80.8	97.6	106.4	120.0	128.9	138.0	145.2	147.7
		53	84.5	101.3	112.3	126.5	135.4	141.9	148.7	150.1
		54	91.2	109.1	120.5	130.3	140.6	149.3	151.4	156.0
		55	86.3	103.5	117.3	129.7	136.1	141.0	144.3	147.5
		56	82.7	101.3	116.0	131.2	144.1	152.4	158.1	161.2
		57	89.2	108.3	123.2	134.8	144.2	151.2	153.0	155.7
		58	81.4	100.8	116.4	128.5	142.2	150.0	154.0	156.5

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
1	f	59	89.7	109.7	126.6	141.0	148.6	160.3	162.1	162.8
		60	97.3	115.6	128.6	136.0	150.4	155.7	161.6	162.0
		61	87.4	105.3	118.8	.	.	.	.	.
		62	96.5	115.3	131.0	.	.	.	.	.
		63	94.1	113.4	126.4	.	.	.	.	.
		64	94.8	112.5	125.4	.	.	.	.	.
		65	84.2	102.9	119.6	.	.	.	.	.
		66	92.6	113.1	128.3	.	.	.	.	.
		67	82.1	102.4	119.2	.	.	.	.	.
		68	92.2	110.7	124.1	.	.	.	.	.
		69	90.5	108.6	125.1	.	.	.	.	.
		70	88.6	110.9	127.7	.	.	.	.	.
		71	93.0	118.5	130.3	.	.	.	.	.
		72	85.8	109.5	126.5	.	.	.	.	.
		73	79.3	101.3	117.0	.	.	.	.	.
		74	86.8	109.1	123.5	.	.	.	.	.
		75	98.3	122.2	139.2	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
2	f	76	82.2	98.9	116.0	.	.	.	.	.
		77	81.6	99.1	116.0	.	.	.	.	.
		78	77.4	92.5	110.8	.	.	.	.	.
		79	84.0	98.2	118.3	.	.	.	.	.
		80	86.0	100.7	119.2	.	.	.	.	.
		81	85.6	101.2	119.4	.	.	.	.	.
		82	79.2	94.9	116.0	.	.	.	.	.
		83	90.2	102.1	122.1	.	.	.	.	.
		84	74.6	87.1	106.9	.	.	.	.	.
		85	76.8	92.2	108.4	.	.	.	.	.
		86	73.6	99.4	116.8	131.0	142.3	147.3	156.3	160.6
		87	67.7	85.8	100.9	110.5	124.8	128.6	134.4	138.8
		88	72.3	97.5	115.2	128.7	140.0	148.6	155.8	165.0
		89	78.8	101.6	119.7	131.6	144.0	148.8	156.0	160.1
		90	81.4	98.6	108.9	115.0	126.6	134.2	141.8	145.9
		91	81.1	109.0	110.5	133.5	143.2	146.9	154.5	157.9
		92	87.2	108.3	109.4	126.1	138.6	143.7	147.6	153.3
		93	85.9	109.3	111.7	131.8	144.7	146.1	149.1	154.3
		94	68.6	83.1	86.0	102.9	116.2	120.1	122.6	129.1
		95	89.5	113.1	114.9	132.8	143.9	149.5	149.9	158.1
		96	74.5	100.0	105.8	111.9	143.3	152.4	152.4	161.6
		97	83.8	102.6	109.5	110.3	139.5	146.8	151.2	156.4
		98	79.9	100.3	106.8	111.6	140.3	149.9	154.7	158.3
		99	88.1	108.9	114.7	116.3	144.4	151.8	153.9	158.9
		100	81.0	101.1	110.1	111.0	132.0	137.3	145.6	152.4
		101	80.3	102.8	100.4	102.5	133.2	144.7	146.2	151.0
		102	84.6	105.0	101.6	103.2	131.2	143.0	144.2	151.3
		103	75.8	101.6	102.6	103.1	136.5	149.3	153.6	159.0
		104	68.3	97.9	96.3	99.4	135.1	145.9	155.5	161.8

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
2	f	105	89.0	111.4	108.0	109.7	140.7	149.4	154.8	160.7
		106	85.2	105.7	110.6	128.0	137.3	144.5	145.5	148.7
		107	84.6	103.3	110.7	127.9	141.0	146.1	150.1	151.7
		108	70.5	90.0	100.3	119.3	131.7	138.4	142.9	144.3
		109	71.0	89.5	99.0	115.9	128.1	133.8	139.5	141.5
		110	69.1	88.6	98.0	117.1	131.8	142.3	147.6	147.1
		111	73.3	103.3	121.0	131.1	140.9	150.9	155.9	158.3
		112	82.6	107.7	119.0	126.5	133.8	142.7	143.8	147.9
		113	82.2	107.1	118.6	124.8	136.5	143.2	142.1	150.9
		114	83.6	114.9	127.7	136.4	142.4	153.9	155.5	160.3
		115	78.1	109.8	123.9	131.4	139.0	149.4	153.8	160.7
		116	69.6	101.4	101.7	126.4	136.5	140.7	147.6	152.3
		117	88.7	113.0	112.2	130.7	139.0	144.2	147.2	152.9
		118	90.6	121.2	121.3	141.5	148.5	157.7	158.5	168.4
		119	79.8	111.9	112.3	133.6	143.7	147.9	152.2	159.0
		120	86.6	117.7	116.7	137.1	147.0	152.0	154.4	162.7
		121	74.1	113.0	114.3	140.5	147.7	159.2	162.1	169.8
		122	78.4	116.0	115.7	138.0	143.7	149.3	154.0	156.5
		123	71.4	105.1	105.2	131.2	136.0	144.4	148.1	153.2
		124	77.2	116.8	118.7	145.3	151.8	160.9	166.5	171.5
		125	90.9	117.5	115.4	138.3	145.3	152.5	152.5	156.1
		126	84.4	100.9	114.2	124.4	136.6	142.8	147.2	152.3
		127	85.8	102.9	119.2	132.5	138.1	144.6	153.3	152.9
		128	82.1	98.1	111.6	123.5	129.2	134.8	138.1	140.5
		129	84.3	100.8	111.1	124.7	133.8	137.6	141.7	146.9
		130	78.6	92.8	107.3	118.0	125.1	132.0	136.7	140.3
		131	90.3	110.8	126.0	137.9	145.8	153.3	158.9	158.5
		132	90.0	109.3	119.6	129.7	138.1	143.7	145.1	149.9
		133	81.0	101.5	112.7	126.6	133.4	140.0	144.0	144.0

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
2	f	134	86.9	109.2	120.7	131.2	141.5	146.4	150.6	154.5
		135	91.8	112.8	119.9	130.6	138.1	145.3	149.1	153.5
		136	95.1	118.0	129.3	.	.	.	.	.
		137	91.4	114.7	126.4	.	.	.	.	.
		138	81.2	103.3	121.9	.	.	.	.	.
		139	87.5	109.7	125.0	.	.	.	.	.
		140	82.6	105.7	118.4	.	.	.	.	.
		141	88.5	108.6	125.9	.	.	.	.	.
		142	96.7	120.9	136.9	.	.	.	.	.
		143	85.9	104.5	118.7	.	.	.	.	.
		144	93.4	116.0	131.3	.	.	.	.	.
		145	92.5	114.7	128.9	.	.	.	.	.
		146	88.1	114.5	129.7	.	.	.	.	.
		147	89.4	111.9	129.6	.	.	.	.	.
		148	97.1	117.9	131.7	.	.	.	.	.
		149	93.9	119.1	138.5	.	.	.	.	.
		150	98.9	124.4	142.1	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
3	f	151	69.0	84.4	99.5	.	.	.	.	.
		152	84.4	102.4	121.0	.	.	.	.	.
		153	81.1	95.9	113.3	.	.	.	.	.
		154	67.7	83.8	98.2	.	.	.	.	.
		155	81.8	97.8	114.6	.	.	.	.	.
		156	77.6	89.7	106.9	.	.	.	.	.
		157	76.8	92.9	111.2	.	.	.	.	.
		158	86.1	103.4	125.1	.	.	.	.	.
		159	82.0	99.6	118.1	.	.	.	.	.
		160	80.6	94.9	112.0	.	.	.	.	.
		161	89.3	111.8	122.3	110.8	144.5	146.2	156.4	160.6
		162	70.8	94.9	110.8	101.0	138.3	141.9	156.4	162.6
		163	88.9	101.6	109.0	99.9	126.5	129.2	136.3	142.4
		164	78.6	93.1	102.9	93.2	118.2	124.8	133.3	140.8
		165	85.8	106.1	118.3	120.3	143.7	146.6	155.1	162.2
		166	74.3	99.5	106.0	127.0	137.7	144.3	152.0	159.2
		167	79.9	102.8	109.0	130.8	143.5	146.4	152.1	159.9
		168	70.1	98.3	102.8	124.0	139.8	141.0	149.9	156.4
		169	82.2	104.7	106.7	124.2	137.2	138.7	143.9	150.0
		170	80.3	100.4	106.9	126.2	138.4	140.3	148.5	155.8
		171	75.2	100.8	107.5	102.7	134.1	146.4	151.7	160.1
		172	81.3	102.3	102.5	97.7	121.6	134.2	135.3	145.1
		173	83.8	106.3	110.3	107.2	135.0	145.6	151.1	158.3
		174	77.2	101.8	106.9	101.4	128.7	139.8	141.6	150.4
		175	85.0	109.2	109.9	105.0	134.6	143.4	146.8	152.8
		176	83.0	103.6	108.0	108.6	136.4	145.3	153.4	159.6
		177	83.3	106.6	108.7	107.7	134.5	139.3	147.5	151.3
		178	79.3	97.7	104.8	109.6	138.6	147.5	153.6	159.7
		179	86.9	109.2	111.5	113.1	141.6	148.7	152.9	158.5

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
3	f	180	72.6	97.3	102.8	106.2	138.3	147.3	154.0	159.8
		181	78.2	100.4	101.1	119.8	130.4	140.6	143.8	144.9
		182	90.9	110.4	109.6	131.2	138.9	148.4	152.4	154.7
		183	85.5	110.0	110.4	133.3	145.0	150.2	155.2	158.1
		184	87.7	108.5	109.0	126.4	137.8	145.0	149.5	150.9
		185	79.8	101.6	102.3	121.7	135.2	141.9	145.3	150.1
		186	88.0	114.2	116.5	134.0	140.4	141.8	149.4	153.2
		187	83.7	112.9	113.9	132.5	139.2	136.9	149.2	153.7
		188	89.6	114.8	117.0	132.7	144.7	144.3	155.9	163.8
		189	68.4	100.5	103.6	127.9	138.0	139.6	149.9	156.2
		190	75.5	104.3	105.7	126.9	137.6	138.9	149.1	153.9
		191	71.5	104.9	117.9	128.9	142.7	147.0	153.1	156.1
		192	90.6	117.7	127.3	132.9	138.9	150.7	153.2	158.7
		193	84.8	113.3	120.8	130.3	137.0	144.9	147.5	152.2
		194	68.1	103.0	119.3	134.0	143.4	153.8	156.6	166.5
		195	74.6	101.7	112.7	123.6	132.2	139.2	143.8	147.0
		196	73.7	110.2	111.8	134.6	143.8	149.9	152.1	151.6
		197	78.3	112.5	111.3	131.0	136.5	146.3	150.4	151.9
		198	90.1	120.0	119.4	134.8	140.4	143.9	152.1	152.6
		199	69.6	110.6	114.5	139.2	142.4	149.8	152.6	156.6
		200	73.0	106.6	107.5	127.9	137.3	143.8	147.3	149.2
		201	97.5	114.5	126.5	142.1	151.0	154.2	164.4	164.9
		202	96.5	113.5	127.0	139.8	150.8	155.0	160.4	165.9
		203	91.8	106.8	119.0	132.7	143.3	148.6	157.9	156.1
		204	80.9	95.2	108.4	122.2	131.1	136.7	142.5	144.9
		205	82.8	97.9	114.3	129.1	142.2	148.7	157.4	158.1
		206	98.1	118.3	131.3	141.1	151.9	154.8	159.6	162.2
		207	88.3	109.9	121.9	136.2	144.5	153.4	154.8	155.5
		208	89.1	107.3	120.4	131.2	143.7	151.1	155.7	159.4

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
3	f	209	93.1	112.7	125.3	131.8	142.4	152.4	155.2	159.1
		210	94.5	114.8	130.1	139.0	149.2	154.0	159.0	158.7
		211	90.2	115.3	129.2	.	.	.	.	.
		212	84.5	109.1	125.7	.	.	.	.	.
		213	87.3	109.9	128.8	.	.	.	.	.
		214	92.2	120.1	132.5	.	.	.	.	.
		215	88.7	110.6	124.9	.	.	.	.	.
		216	87.2	108.9	127.5	.	.	.	.	.
		217	81.5	106.2	124.7	.	.	.	.	.
		218	85.0	106.3	125.0	.	.	.	.	.
		219	93.9	113.7	126.8	.	.	.	.	.
		220	86.4	107.1	119.3	.	.	.	.	.
		221	92.6	116.7	133.2	.	.	.	.	.
		222	90.5	110.3	128.5	.	.	.	.	.
		223	82.6	104.9	119.4	.	.	.	.	.
		224	78.6	103.1	120.7	.	.	.	.	.
		225	84.2	110.6	126.0	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
4	f	226	70.9	88.2	107.1	.	.	.	.	.
		227	82.6	94.6	114.5	.	.	.	.	.
		228	87.6	100.4	116.5	.	.	.	.	.
		229	67.9	86.9	105.5	.	.	.	.	.
		230	90.6	107.3	124.2	.	.	.	.	.
		231	75.5	86.4	105.9	.	.	.	.	.
		232	69.0	84.5	103.9	.	.	.	.	.
		233	81.1	96.8	117.7	.	.	.	.	.
		234	73.3	88.3	110.5	.	.	.	.	.
		235	72.3	87.8	108.9	.	.	.	.	.
		236	80.0	102.2	115.8	126.3	135.4	139.0	147.1	153.4
		237	77.1	96.0	107.6	118.4	128.1	132.6	141.9	146.8
		238	81.8	102.0	113.7	126.1	135.8	143.1	151.7	156.1
		239	76.8	94.5	113.2	124.6	137.7	139.2	150.1	153.7
		240	86.1	105.9	121.5	134.0	137.4	147.7	151.2	157.3
		241	90.0	112.2	124.3	129.9	144.9	146.9	155.1	160.8
		242	77.5	103.7	121.4	136.7	150.5	153.6	160.1	164.4
		243	74.1	95.0	111.9	122.5	134.4	142.3	149.2	151.9
		244	80.1	104.7	119.0	129.5	140.1	146.2	150.3	158.1
		245	90.3	110.4	122.2	130.5	142.9	148.5	148.7	154.9
		246	68.3	91.2	90.8	99.3	129.4	136.4	141.9	147.1
		247	85.3	105.2	102.1	105.6	133.9	147.1	149.5	151.7
		248	70.0	97.0	97.7	105.2	139.2	146.4	156.0	157.8
		249	80.6	102.1	99.5	103.4	132.9	143.7	148.4	152.5
		250	78.2	102.7	100.2	106.3	134.4	142.9	150.7	158.0
		251	73.4	95.4	101.1	124.0	139.9	148.9	155.0	158.3
		252	71.7	93.7	97.8	121.8	134.8	145.7	152.2	155.0
		253	69.5	94.3	98.0	124.4	137.8	149.5	154.9	158.6
		254	74.3	95.9	102.2	122.1	134.2	142.7	147.7	149.7

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
4	f	255	74.9	99.3	104.4	125.3	135.9	142.8	148.2	150.7
		256	83.1	104.7	108.4	125.7	137.5	146.5	149.9	153.5
		257	83.7	110.3	111.0	128.3	139.5	146.7	151.0	155.0
		258	78.7	101.3	105.7	125.8	136.6	141.4	147.3	148.8
		259	82.9	106.4	107.5	122.9	135.7	142.1	144.7	146.1
		260	84.7	104.7	104.4	118.4	127.6	134.3	138.3	138.3
		261	85.9	110.3	113.9	127.5	135.7	141.0	147.4	151.9
		262	83.9	112.5	116.7	129.6	138.2	144.9	145.6	150.7
		263	79.0	110.6	117.9	136.2	143.1	153.7	159.0	163.9
		264	89.2	116.8	121.1	134.7	143.9	150.6	150.9	159.1
		265	84.0	117.4	125.5	138.7	145.0	154.3	156.3	162.5
		266	79.6	107.0	107.3	130.5	134.3	140.6	145.4	148.8
		267	90.9	115.1	114.1	132.5	141.8	150.9	152.5	158.9
		268	88.3	113.1	108.1	128.0	137.3	145.2	150.5	152.8
		269	68.7	99.4	103.1	130.3	139.1	151.3	154.6	160.7
		270	78.4	108.6	105.4	126.3	133.1	143.2	147.8	154.4
		271	85.6	118.9	130.8	139.7	148.3	151.4	156.9	160.2
		272	82.2	112.8	125.2	137.4	146.5	151.9	159.0	161.1
		273	88.7	123.5	132.7	141.4	150.2	156.9	160.4	164.7
		274	81.5	113.9	124.8	132.8	138.5	144.9	151.7	154.1
		275	87.1	122.2	132.1	139.9	147.2	153.7	155.4	161.0
		276	92.4	107.4	124.4	135.0	145.8	152.8	154.1	159.5
		277	81.2	97.6	113.8	126.4	131.0	137.8	141.5	145.5
		278	80.5	98.4	113.7	127.0	134.6	141.1	145.8	148.9
		279	93.5	108.9	123.7	137.0	146.3	151.7	157.5	159.6
		280	87.5	101.5	115.9	129.1	134.4	142.1	147.6	151.5
		281	86.5	104.6	115.3	124.3	128.8	138.1	140.9	142.9
		282	88.4	108.0	124.6	135.4	145.0	152.8	157.0	162.3
		283	89.7	110.4	122.6	132.8	142.6	151.1	154.0	158.9

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
4	f	284	84.3	102.9	116.6	130.6	137.7	148.2	150.3	152.0
		285	90.3	108.8	117.5	130.2	138.0	143.8	149.1	146.5
		286	83.0	106.7	122.9	.	.	.	.	.
		287	84.4	108.1	122.2	.	.	.	.	.
		288	96.4	119.2	131.7	.	.	.	.	.
		289	94.5	114.2	126.2	.	.	.	.	.
		290	85.8	110.9	130.4	.	.	.	.	.
		291	87.0	109.1	127.7	.	.	.	.	.
		292	90.8	112.7	129.1	.	.	.	.	.
		293	93.7	115.9	135.0	.	.	.	.	.
		294	78.4	98.5	116.0	.	.	.	.	.
		295	82.6	103.5	119.0	.	.	.	.	.
		296	91.8	118.4	132.3	.	.	.	.	.
		297	88.9	108.1	121.6	.	.	.	.	.
		298	88.8	115.4	131.4	.	.	.	.	.
		299	96.9	120.2	131.1	.	.	.	.	.
		300	97.8	122.2	140.8	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
5	f	301	82.7	97.2	114.3	.	.	.	.	.
		302	72.8	90.0	106.2	.	.	.	.	.
		303	86.6	102.1	119.0	.	.	.	.	.
		304	84.9	100.2	116.6	.	.	.	.	.
		305	77.7	93.7	116.5	.	.	.	.	.
		306	75.4	89.5	107.7	.	.	.	.	.
		307	88.7	100.1	116.0	.	.	.	.	.
		308	70.8	86.7	105.6	.	.	.	.	.
		309	68.3	86.8	105.2	.	.	.	.	.
		310	87.7	101.3	113.2	.	.	.	.	.
		311	85.6	104.8	111.6	122.1	131.6	140.1	144.9	151.5
		312	69.4	87.3	95.6	112.6	119.9	126.9	132.4	137.9
		313	76.8	98.8	109.9	122.9	133.8	138.1	145.6	153.2
		314	78.8	98.8	108.3	124.0	132.8	139.2	142.5	149.3
		315	70.6	90.6	101.1	113.5	126.5	133.3	135.1	145.9
		316	78.4	100.1	108.2	115.9	128.1	137.4	142.6	147.3
		317	82.1	100.2	111.8	121.1	138.6	143.6	149.7	152.3
		318	84.3	106.8	120.5	130.5	143.4	142.0	150.6	154.3
		319	80.1	104.0	117.7	127.8	143.5	141.9	150.5	156.0
		320	74.4	99.3	111.6	120.8	134.6	141.9	145.9	151.4
		321	89.2	109.1	118.1	105.1	140.8	154.4	162.8	165.8
		322	89.7	114.0	122.1	110.3	141.3	154.3	157.9	161.5
		323	88.6	111.4	116.1	104.1	133.6	145.9	150.2	151.3
		324	81.2	106.2	117.0	105.7	136.9	151.5	155.5	158.5
		325	74.3	94.2	102.2	92.8	122.2	135.3	143.1	145.6
		326	90.2	109.8	109.6	106.2	135.2	147.1	153.7	156.4
		327	85.9	110.1	111.3	109.8	139.6	149.0	156.5	158.0
		328	80.1	100.1	99.7	97.9	125.0	134.1	138.0	139.4
		329	81.1	106.7	107.1	106.6	138.2	148.0	155.2	154.7

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
5	f	330	77.0	102.5	104.9	107.2	137.3	148.3	151.9	157.1
		331	83.6	105.3	111.5	126.8	137.5	147.4	152.9	158.9
		332	67.7	96.1	105.6	122.2	137.5	140.4	147.1	150.2
		333	83.8	105.6	109.0	118.1	127.6	132.7	133.9	136.2
		334	82.2	106.9	112.7	127.3	140.2	144.4	145.6	148.0
		335	86.0	107.1	112.3	127.8	138.9	144.6	147.3	148.6
		336	90.5	119.8	130.2	141.6	148.9	156.6	157.9	163.7
		337	79.8	111.5	121.7	136.3	147.3	153.5	156.8	159.9
		338	69.0	101.4	110.5	119.0	127.6	132.0	133.3	140.0
		339	90.7	114.9	120.0	129.5	137.5	139.6	140.3	149.4
		340	82.0	114.8	125.0	135.3	140.4	148.5	151.0	156.8
		341	83.9	115.1	122.6	129.4	137.5	145.1	148.7	152.7
		342	68.3	100.8	114.9	128.8	136.9	145.8	149.2	155.1
		343	73.2	109.6	122.2	133.1	143.4	151.4	153.5	160.5
		344	84.9	118.7	132.4	137.0	146.4	156.1	158.5	164.3
		345	72.1	102.1	114.6	123.5	131.2	137.4	141.5	146.5
		346	75.2	109.6	104.4	126.8	135.6	144.0	148.4	150.3
		347	79.4	113.4	110.6	135.8	142.6	146.0	150.8	153.2
		348	80.3	117.1	110.9	136.8	144.2	148.3	155.2	158.3
		349	77.6	114.2	109.1	130.4	139.6	146.1	150.1	152.7
		350	73.4	110.5	105.2	126.6	135.4	148.9	150.7	155.2
		351	84.9	102.0	115.3	130.1	136.2	143.7	149.0	150.9
		352	95.2	108.9	117.6	130.7	135.7	136.5	144.9	144.9
		353	82.2	95.7	107.7	119.6	127.6	131.7	138.8	139.9
		354	97.2	108.2	119.5	132.1	141.1	145.5	153.0	152.6
		355	93.0	104.3	114.6	127.3	134.6	141.4	144.4	149.7
		356	87.1	103.8	111.4	122.1	130.6	138.0	142.1	143.2
		357	78.7	99.3	115.5	127.9	138.7	145.7	146.6	151.8
		358	92.0	110.8	126.1	135.4	146.6	152.2	157.2	158.8

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
5	f	359	90.6	110.6	122.0	133.7	140.5	148.2	153.2	151.4
		360	83.1	103.9	116.5	126.5	136.3	142.4	147.3	148.3
		361	87.9	110.4	122.3	.	.	.	.	.
		362	87.3	110.2	125.3	.	.	.	.	.
		363	89.7	112.7	128.4	.	.	.	.	.
		364	90.8	112.9	123.6	.	.	.	.	.
		365	92.7	112.5	124.3	.	.	.	.	.
		366	86.5	108.8	123.1	.	.	.	.	.
		367	89.0	112.8	126.2	.	.	.	.	.
		368	85.0	107.1	122.2	.	.	.	.	.
		369	88.9	110.7	120.8	.	.	.	.	.
		370	94.9	117.5	129.1	.	.	.	.	.
		371	94.4	119.7	125.5	.	.	.	.	.
		372	82.7	106.2	105.5	.	.	.	.	.
		373	80.8	108.3	110.4	.	.	.	.	.
		374	81.7	104.6	109.1	.	.	.	.	.
		375	97.9	123.3	123.6	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
6	f	376	71.1	86.9	106.0	.	.	.	.	.
		377	85.6	105.0	119.6	.	.	.	.	.
		378	75.3	88.7	103.2	.	.	.	.	.
		379	73.1	87.5	103.9	.	.	.	.	.
		380	81.2	100.0	116.7	.	.	.	.	.
		381	83.8	98.4	116.2	.	.	.	.	.
		382	78.5	93.7	107.6	.	.	.	.	.
		383	77.3	92.3	109.0	.	.	.	.	.
		384	85.8	99.6	115.8	.	.	.	.	.
		385	82.2	101.7	116.0	.	.	.	.	.
		386	90.8	107.6	114.1	112.1	132.0	139.5	143.8	146.8
		387	83.9	101.2	112.8	112.3	129.2	137.3	143.9	147.2
		388	77.9	94.9	102.6	104.1	122.8	129.3	136.7	141.0
		389	76.0	95.2	104.0	106.3	120.1	129.0	136.0	139.7
		390	68.7	90.5	103.4	108.9	128.5	141.0	149.5	154.5
		391	74.6	99.3	100.4	123.7	135.3	140.9	144.9	151.2
		392	89.7	111.1	105.7	124.4	135.1	139.9	143.2	148.7
		393	69.8	89.0	89.3	107.8	120.8	127.0	132.3	138.3
		394	76.6	97.3	96.4	116.4	130.0	134.3	139.6	145.0
		395	80.3	102.6	102.0	121.4	134.8	141.2	146.0	149.6
		396	85.4	102.0	113.3	113.3	134.4	141.8	146.0	148.7
		397	80.0	107.4	120.0	119.8	144.1	157.2	162.8	164.8
		398	90.6	110.0	117.0	114.5	134.4	145.5	149.0	150.2
		399	84.2	104.5	115.2	116.0	135.7	144.1	148.2	155.6
		400	88.7	110.6	120.0	121.1	145.1	154.0	158.1	163.4
		401	81.9	103.5	97.6	119.6	132.1	141.0	142.8	145.2
		402	90.3	113.2	105.3	126.1	140.6	147.4	152.1	153.1
		403	86.3	108.8	104.8	125.5	138.0	143.4	152.8	156.3
		404	80.4	104.4	100.6	123.0	137.0	144.9	146.1	152.2

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
6	f	405	87.9	117.1	113.2	139.9	159.2	168.8	173.8	178.9
		406	68.9	91.8	102.0	115.4	126.3	135.5	138.9	140.7
		407	68.1	96.0	110.8	125.3	137.9	145.3	151.9	153.1
		408	79.3	104.8	118.6	130.9	145.9	152.0	155.7	155.6
		409	70.0	91.1	102.4	112.1	124.2	132.4	134.9	138.0
		410	74.1	102.0	116.3	129.9	139.2	146.3	152.0	149.7
		411	83.3	108.5	122.0	128.9	140.6	146.4	151.4	156.5
		412	71.7	95.5	109.7	116.3	124.8	130.1	133.1	139.2
		413	78.9	109.1	119.9	127.0	135.4	145.8	147.5	155.2
		414	86.1	115.4	123.4	130.3	140.0	145.4	144.8	151.9
		415	72.2	101.9	114.2	122.7	132.4	138.8	142.7	145.8
		416	87.3	116.4	122.7	132.7	140.1	147.9	149.4	154.0
		417	81.3	114.5	121.4	131.6	141.2	147.9	152.1	160.8
		418	68.2	102.5	110.4	124.7	133.8	138.8	143.3	146.4
		419	82.1	111.1	115.3	125.7	134.8	138.2	141.2	144.1
		420	82.8	107.8	116.0	122.9	135.2	144.3	147.5	152.0
		421	89.5	118.6	124.8	132.2	137.8	144.4	150.0	153.2
		422	84.6	114.7	124.1	131.9	135.0	139.7	145.1	150.1
		423	77.7	114.8	123.4	135.1	144.6	153.1	157.1	158.6
		424	79.4	112.6	121.4	129.4	136.2	145.6	148.2	153.2
		425	73.5	107.3	116.9	128.3	132.3	138.3	143.8	146.7
		426	89.4	103.0	113.0	123.2	135.4	141.8	148.0	148.0
		427	88.3	102.3	115.4	127.3	136.3	142.4	148.5	149.1
		428	89.4	101.1	112.9	125.2	134.7	143.6	145.9	147.2
		429	86.4	100.1	110.4	124.7	132.0	138.3	142.5	142.8
		430	85.9	100.5	116.1	129.4	140.1	146.4	154.5	153.9
		431	82.8	101.3	110.9	122.4	131.0	138.5	141.3	141.0
		432	87.4	106.3	116.8	129.8	138.3	147.9	151.4	152.3
		433	84.1	102.1	113.6	125.6	137.4	146.5	151.2	152.6

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

Day numbers relative to Start Date

Group	Sex	Animal	Week -1	1	8	15	22	29	36	43
6	f	434	82.5	101.7	113.8	125.0	135.5	141.5	148.5	150.3
		435	90.3	109.4	118.8	132.4	141.8	150.4	151.9	154.8
		436	93.3	121.9	136.5	.	.	.	.	.
		437	90.9	112.2	122.6	.	.	.	.	.
		438	88.6	108.1	120.3	.	.	.	.	.
		439	81.3	105.4	119.7	.	.	.	.	.
		440	79.6	101.4	112.9	.	.	.	.	.
		441	92.3	105.7	116.0	.	.	.	.	.
		442	80.7	99.7	113.4	.	.	.	.	.
		443	87.2	107.4	119.9	.	.	.	.	.
		444	95.4	110.9	120.6	.	.	.	.	.
		445	97.7	122.2	131.9	.	.	.	.	.
		446	92.9	118.9	122.1	.	.	.	.	.
		447	95.2	121.3	117.6	.	.	.	.	.
		448	94.3	113.5	113.3	.	.	.	.	.
		449	84.8	106.3	110.7	.	.	.	.	.
		450	99.0	126.4	124.7	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
1	f	1	.	.	.	.	.	.	.
		2	.	.	.	.	.	.	.
		3	.	.	.	.	.	.	.
		4	.	.	.	.	.	.	.
		5	.	.	.	.	.	.	.
		6	.	.	.	.	.	.	.
		7	.	.	.	.	.	.	.
		8	.	.	.	.	.	.	.
		9	.	.	.	.	.	.	.
		10	.	.	.	.	.	.	.
		11	134.4	159.3	159.6	164.1	163.6	168.4	171.4
		12	138.8	160.8	161.1	171.1	169.2	174.9	179.7
		13	150.7	176.2	175.6	182.0	185.1	187.7	192.9
		14	122.3	142.5	147.4	153.6	153.9	158.8	163.1
		15	136.4	161.0	162.0	170.5	172.3	177.2	180.8
		16	158.1	157.8	160.3	169.0	165.6	171.5	174.7
		17	150.0	150.7	152.4	160.0	157.9	162.8	164.2
		18	163.8	162.0	166.7	173.0	169.5	173.6	178.3
		19	166.8	167.6	170.2	176.1	176.6	178.9	183.8
		20	166.1	166.4	167.0	174.3	170.8	176.7	180.9
		21	158.2	165.3	165.6	167.9	174.4	175.4	182.4
		22	151.9	161.5	161.2	168.9	169.8	173.7	179.2
		23	146.0	148.9	150.2	155.9	153.7	160.1	162.2
		24	149.5	155.8	154.5	162.0	165.6	169.5	174.9
		25	154.6	159.3	162.7	170.4	167.3	176.5	183.5
		26	161.2	171.0	173.0	177.7	181.0	184.4	186.1
		27	151.2	156.0	160.6	162.4	164.8	172.2	174.4
		28	148.6	154.4	156.4	158.9	163.5	166.2	171.4
		29	156.0	159.0	161.6	171.8	171.7	184.2	180.1

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
1	f	30	148.3	155.2	154.2	161.6	161.1	167.7	168.3
		31	157.4	160.2	161.9	167.3	170.1	174.1	180.4
		32	160.7	161.5	167.6	170.0	170.9	176.2	182.9
		33	153.8	155.8	157.9	162.3	167.1	170.2	175.9
		34	155.7	156.6	160.0	167.3	168.9	171.6	179.3
		35	148.4	150.7	153.5	158.1	157.9	165.4	169.0
		36	159.2	169.4	166.3	170.1	172.7	171.8	176.8
		37	156.6	163.7	162.7	167.8	169.2	167.0	181.4
		38	159.0	162.5	162.3	166.6	172.9	171.1	178.4
		39	157.6	163.0	160.6	168.9	169.7	171.6	181.8
		40	168.9	176.5	174.6	179.9	178.7	180.5	183.9
		41	171.1	177.2	176.9	180.4	184.1	183.3	188.7
		42	160.6	164.5	161.2	168.8	168.4	169.9	176.5
		43	165.4	169.9	171.3	175.9	174.7	177.7	184.2
		44	155.9	162.3	159.3	164.4	164.3	163.5	168.9
		45	153.6	157.0	158.9	163.6	162.4	162.7	169.0
		46	151.8	153.5	158.4	158.1	162.8	161.9	171.1
		47	161.7	162.0	166.7	169.5	172.6	175.4	179.6
		48	153.1	155.0	161.5	162.0	164.5	167.2	171.2
		49	157.3	154.6	162.4	163.6	168.3	169.7	172.6
		50	155.3	158.0	163.5	165.7	168.8	171.2	173.4
		51	163.6	166.2	172.0	171.1	172.5	181.0	182.8
		52	152.5	154.3	158.5	165.3	161.9	167.2	170.7
		53	160.6	162.8	168.2	172.1	173.3	176.0	182.4
		54	156.2	161.8	167.8	169.4	168.8	176.0	175.1
		55	153.1	152.7	161.2	162.7	161.0	169.3	171.5
		56	168.6	171.3	173.1	176.9	177.3	181.1	187.2
		57	162.3	162.7	164.5	166.5	170.3	169.6	173.0
		58	163.7	165.7	168.0	170.9	178.1	176.9	183.3

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
1	f	59	171.5	171.2	179.2	183.3	186.3	189.6	197.1
		60	170.6	172.9	172.5	176.7	180.4	184.1	190.1
		61	.	.	.	.	.	.	.
		62	.	.	.	.	.	.	.
		63	.	.	.	.	.	.	.
		64	.	.	.	.	.	.	.
		65	.	.	.	.	.	.	.
		66	.	.	.	.	.	.	.
		67	.	.	.	.	.	.	.
		68	.	.	.	.	.	.	.
		69	.	.	.	.	.	.	.
		70	.	.	.	.	.	.	.
		71	.	.	.	.	.	.	.
		72	.	.	.	.	.	.	.
		73	.	.	.	.	.	.	.
		74	.	.	.	.	.	.	.
		75	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
2	f	76	.	.	.	.	.	.	.
		77	.	.	.	.	.	.	.
		78	.	.	.	.	.	.	.
		79	.	.	.	.	.	.	.
		80	.	.	.	.	.	.	.
		81	.	.	.	.	.	.	.
		82	.	.	.	.	.	.	.
		83	.	.	.	.	.	.	.
		84	.	.	.	.	.	.	.
		85	.	.	.	.	.	.	.
		86	165.2	168.7	171.8	176.7	182.3	187.8	189.7
		87	141.0	144.8	145.8	150.6	154.4	153.9	160.0
		88	168.1	174.4	174.0	183.0	181.0	185.0	192.9
		89	168.3	170.1	172.3	175.5	179.4	185.0	187.3
		90	152.5	154.7	158.2	166.6	166.9	170.2	177.6
		91	166.1	166.5	163.9	178.0	172.6	175.9	182.8
		92	158.6	159.2	161.5	170.9	166.6	174.4	177.7
		93	159.5	163.0	160.8	173.0	171.7	174.7	181.9
		94	135.3	137.6	138.4	142.1	137.5	142.8	147.2
		95	164.5	167.2	169.5	174.9	175.0	173.7	180.9
		96	161.6	168.1	172.6	175.5	183.1	190.1	187.7
		97	156.5	163.4	161.9	170.2	167.7	177.6	183.2
		98	161.9	167.0	172.0	178.2	178.6	185.0	186.8
		99	162.3	165.8	167.8	176.0	175.1	181.3	183.9
		100	153.7	158.1	163.1	165.4	169.8	170.7	176.4
		101	149.3	153.7	158.7	159.5	157.8	165.3	167.5
		102	149.6	155.3	158.3	161.1	162.3	166.5	169.7
		103	161.8	169.1	171.6	178.7	172.3	181.7	185.3
		104	160.4	167.0	171.2	171.6	168.5	178.2	182.6

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
2	f	105	158.2	165.8	167.8	175.9	167.9	181.4	184.1
		106	153.2	151.6	157.1	156.0	161.5	166.7	171.7
		107	156.7	159.9	161.8	165.8	165.7	172.1	175.3
		108	149.8	152.1	157.4	156.9	164.7	166.4	171.0
		109	146.0	150.0	150.6	155.0	157.5	163.1	166.5
		110	153.6	154.3	157.4	161.0	165.8	170.7	177.2
		111	160.8	168.3	164.9	174.3	168.5	174.2	179.4
		112	153.5	156.5	155.7	159.5	160.0	162.3	168.2
		113	150.3	157.5	157.6	160.4	163.4	163.7	168.2
		114	165.6	169.5	171.5	172.3	173.4	175.1	183.6
		115	158.4	166.7	166.5	169.9	176.2	170.4	179.1
		116	155.4	158.5	155.4	167.0	167.9	168.7	178.6
		117	160.3	161.1	160.7	168.8	169.0	172.1	175.6
		118	168.7	173.5	175.9	184.3	183.5	185.6	194.2
		119	163.6	165.7	164.7	172.5	172.2	172.6	178.3
		120	160.8	168.4	166.0	179.5	176.5	180.4	184.0
		121	171.7	174.8	181.1	180.5	181.3	183.6	182.7
		122	158.6	157.9	161.6	164.4	168.0	173.0	174.3
		123	154.2	154.4	161.3	162.2	167.9	170.7	170.9
		124	166.9	168.8	177.6	173.4	180.0	180.5	182.2
		125	156.3	156.7	161.1	162.0	167.6	168.5	170.3
		126	156.1	162.4	166.5	158.0	167.5	171.4	171.6
		127	165.0	162.3	167.1	173.6	170.0	180.6	181.5
		128	148.1	148.4	155.1	157.9	158.1	160.3	161.7
		129	152.9	153.8	157.6	163.2	161.5	165.0	165.9
		130	145.5	146.1	149.9	152.0	151.1	155.9	158.2
		131	171.0	166.8	170.9	176.9	179.5	181.3	188.1
		132	156.8	155.4	157.1	159.9	165.7	165.1	170.9
		133	155.7	156.4	160.9	164.7	165.9	169.1	171.0

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
2	f	134	159.4	159.5	162.3	166.5	170.7	169.8	175.8
		135	164.2	161.3	162.8	168.8	172.3	173.8	175.2
		136	.	.	.	.	.	.	.
		137	.	.	.	.	.	.	.
		138	.	.	.	.	.	.	.
		139	.	.	.	.	.	.	.
		140	.	.	.	.	.	.	.
		141	.	.	.	.	.	.	.
		142	.	.	.	.	.	.	.
		143	.	.	.	.	.	.	.
		144	.	.	.	.	.	.	.
		145	.	.	.	.	.	.	.
		146	.	.	.	.	.	.	.
		147	.	.	.	.	.	.	.
		148	.	.	.	.	.	.	.
		149	.	.	.	.	.	.	.
		150	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
3	f	151	.	.	.	.	.	.	.
		152	.	.	.	.	.	.	.
		153	.	.	.	.	.	.	.
		154	.	.	.	.	.	.	.
		155	.	.	.	.	.	.	.
		156	.	.	.	.	.	.	.
		157	.	.	.	.	.	.	.
		158	.	.	.	.	.	.	.
		159	.	.	.	.	.	.	.
		160	.	.	.	.	.	.	.
		161	166.2	168.6	169.3	179.3	172.5	181.8	160.2
		162	164.7	170.8	170.6	177.6	180.3	185.2	165.9
		163	145.9	148.6	150.1	155.1	156.2	158.7	141.8
		164	143.7	148.6	151.8	155.8	158.1	160.2	141.7
		165	165.3	168.7	173.1	177.6	178.8	184.1	166.4
		166	162.1	169.0	165.8	175.5	173.6	177.2	182.6
		167	162.1	166.7	168.7	177.0	175.2	177.1	183.2
		168	162.5	163.6	164.1	175.8	173.6	178.7	186.8
		169	155.6	159.7	161.1	168.2	169.3	172.1	177.8
		170	160.2	160.6	165.7	170.1	177.3	180.4	183.0
		171	162.4	168.0	168.9	174.9	175.7	177.6	184.5
		172	144.0	152.7	152.7	158.3	156.3	160.8	162.1
		173	161.2	168.4	167.5	174.5	174.1	180.2	183.9
		174	148.0	156.9	156.0	174.6	162.8	165.8	171.3
		175	150.4	160.4	159.4	162.8	164.5	166.5	173.7
		176	158.8	163.5	170.2	173.1	176.8	184.1	183.1
		177	151.0	156.4	157.5	173.8	167.5	172.6	176.3
		178	155.3	163.8	167.0	171.7	174.8	178.7	183.5
		179	158.5	164.5	166.4	173.1	173.6	178.9	180.8

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
3	f	180	157.7	166.0	167.4	173.3	177.4	184.7	186.5
		181	150.9	149.5	158.3	159.5	162.7	169.9	170.4
		182	158.4	155.5	160.8	162.4	169.7	175.5	177.2
		183	162.2	163.3	164.1	170.2	173.1	175.5	182.4
		184	154.9	154.5	158.4	162.0	163.9	171.1	173.8
		185	153.2	156.2	160.0	161.7	169.2	168.2	174.0
		186	156.3	159.4	159.6	164.3	163.9	165.6	172.9
		187	156.9	164.9	159.3	168.1	165.8	167.7	174.0
		188	166.3	173.6	170.4	180.8	178.9	180.1	190.5
		189	158.6	163.8	163.4	167.9	167.8	169.9	174.1
		190	156.8	165.6	162.2	169.6	167.9	169.2	174.3
		191	160.4	168.1	163.1	172.5	170.0	170.0	178.1
		192	161.6	166.7	167.2	173.0	171.1	173.8	178.5
		193	154.3	156.1	158.0	161.7	159.4	161.4	167.2
		194	164.3	175.0	173.0	180.5	184.8	184.6	187.6
		195	153.4	159.7	158.6	166.5	164.5	166.6	173.2
		196	158.7	159.4	165.9	165.7	165.9	174.9	176.6
		197	155.5	155.6	160.2	161.8	161.6	170.0	171.9
		198	156.5	157.8	162.4	162.4	165.4	166.9	171.4
		199	156.8	159.8	165.7	164.5	171.7	176.2	176.6
		200	148.4	153.1	156.3	155.6	159.7	161.0	162.2
		201	170.5	175.7	179.0	184.5	178.9	184.8	183.7
		202	174.0	177.2	187.0	191.0	188.2	194.0	197.1
		203	164.0	166.8	172.9	176.8	175.4	181.8	186.3
		204	154.3	153.4	157.6	161.9	159.4	166.4	164.5
		205	164.5	166.1	169.4	174.7	170.2	172.3	175.1
		206	170.4	172.2	172.2	178.6	181.8	184.8	190.8
		207	167.7	167.4	168.4	174.6	178.0	181.0	185.2
		208	169.6	169.9	173.5	176.8	180.7	185.3	192.4

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
3	f	209	170.3	169.8	175.6	175.7	180.2	178.3	179.3
		210	167.3	165.2	171.3	169.4	174.1	176.8	181.5
		211	.	.	.	.	.	.	.
		212	.	.	.	.	.	.	.
		213	.	.	.	.	.	.	.
		214	.	.	.	.	.	.	.
		215	.	.	.	.	.	.	.
		216	.	.	.	.	.	.	.
		217	.	.	.	.	.	.	.
		218	.	.	.	.	.	.	.
		219	.	.	.	.	.	.	.
		220	.	.	.	.	.	.	.
		221	.	.	.	.	.	.	.
		222	.	.	.	.	.	.	.
		223	.	.	.	.	.	.	.
		224	.	.	.	.	.	.	.
		225	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
4	f	226	.	.	.	.	.	.	.
		227	.	.	.	.	.	.	.
		228	.	.	.	.	.	.	.
		229	.	.	.	.	.	.	.
		230	.	.	.	.	.	.	.
		231	.	.	.	.	.	.	.
		232	.	.	.	.	.	.	.
		233	.	.	.	.	.	.	.
		234	.	.	.	.	.	.	.
		235	.	.	.	.	.	.	.
		236	154.8	156.0	160.0	165.3	165.2	170.3	171.8
		237	149.2	153.5	157.4	165.5	165.2	166.2	171.2
		238	159.2	161.0	164.3	172.2	170.0	175.7	175.8
		239	157.2	164.7	162.6	173.1	171.7	176.3	180.4
		240	160.9	161.9	167.8	174.2	174.3	181.0	183.4
		241	164.1	169.4	170.1	178.4	175.6	179.8	182.1
		242	171.9	175.2	175.7	169.7	179.8	188.4	193.4
		243	158.4	159.1	160.8	171.5	171.1	175.5	177.5
		244	162.3	162.6	165.8	173.4	173.7	177.5	181.1
		245	157.8	164.6	161.6	169.7	169.7	171.3	176.9
		246	147.6	152.8	157.0	162.3	165.7	166.7	170.8
		247	153.8	158.6	163.1	166.5	168.0	169.7	170.4
		248	163.5	169.3	172.5	181.0	180.7	183.7	191.2
		249	152.2	154.8	161.9	164.1	164.6	169.7	170.0
		250	157.6	163.7	167.8	170.7	173.5	177.3	177.4
		251	160.9	168.9	170.6	174.9	174.7	181.8	183.8
		252	155.8	164.0	164.4	167.4	166.7	172.1	175.9
		253	164.7	171.7	171.4	177.9	178.0	186.0	192.0
		254	152.2	158.7	162.7	166.2	168.3	172.8	174.8

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
4	f	255	154.5	159.1	161.9	167.2	166.1	172.8	175.0
		256	158.5	160.9	164.1	166.2	170.0	175.9	178.2
		257	157.8	159.5	164.3	164.8	170.0	174.8	177.2
		258	154.0	157.0	157.6	161.3	162.4	169.2	172.5
		259	151.0	151.9	155.5	160.0	161.7	167.2	170.8
		260	141.0	143.9	148.8	150.5	153.3	157.4	159.3
		261	154.2	162.3	161.8	166.8	166.1	169.7	172.3
		262	151.9	160.5	157.6	162.2	164.4	165.4	170.2
		263	169.8	175.8	175.7	180.1	180.1	184.5	186.5
		264	157.7	166.2	162.4	170.6	170.5	173.1	177.6
		265	166.9	172.3	173.2	175.2	174.6	180.9	182.0
		266	152.2	159.4	154.6	163.7	160.4	163.9	167.6
		267	159.1	168.8	165.9	170.7	171.3	174.8	178.3
		268	158.7	164.7	163.6	173.1	168.5	175.7	180.5
		269	161.3	165.9	163.9	166.8	168.1	172.3	176.4
		270	157.2	162.2	160.7	164.7	166.3	168.5	172.8
		271	161.9	163.7	166.9	171.0	172.0	173.1	173.2
		272	161.1	164.3	168.5	170.7	171.9	176.7	180.1
		273	167.3	167.9	175.2	174.2	177.3	178.0	181.5
		274	156.1	157.9	164.4	163.9	165.9	170.7	175.5
		275	162.9	166.3	176.6	172.7	175.4	179.5	178.5
		276	167.7	167.1	174.6	174.2	177.2	183.7	184.4
		277	152.5	151.7	160.2	161.9	163.1	171.6	174.3
		278	158.0	157.1	161.3	162.2	163.0	167.5	168.2
		279	167.9	170.2	172.8	177.0	176.9	183.1	184.7
		280	159.0	157.4	164.3	166.5	166.1	177.7	178.2
		281	150.7	151.3	154.8	157.3	160.2	160.9	163.5
		282	168.1	172.5	177.8	177.2	183.8	180.9	185.0
		283	167.1	168.2	170.3	174.8	175.9	173.5	179.7

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
4	f	284	162.2	162.8	166.9	170.7	172.9	174.9	180.3
		285	157.4	157.7	162.9	169.8	169.7	173.8	177.1
		286	.	.	.	.	.	.	.
		287	.	.	.	.	.	.	.
		288	.	.	.	.	.	.	.
		289	.	.	.	.	.	.	.
		290	.	.	.	.	.	.	.
		291	.	.	.	.	.	.	.
		292	.	.	.	.	.	.	.
		293	.	.	.	.	.	.	.
		294	.	.	.	.	.	.	.
		295	.	.	.	.	.	.	.
		296	.	.	.	.	.	.	.
		297	.	.	.	.	.	.	.
		298	.	.	.	.	.	.	.
		299	.	.	.	.	.	.	.
		300	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
5	f	301	.	.	.	.	.	.	.
		302	.	.	.	.	.	.	.
		303	.	.	.	.	.	.	.
		304	.	.	.	.	.	.	.
		305	.	.	.	.	.	.	.
		306	.	.	.	.	.	.	.
		307	.	.	.	.	.	.	.
		308	.	.	.	.	.	.	.
		309	.	.	.	.	.	.	.
		310	.	.	.	.	.	.	.
		311	157.0	160.0	163.3	170.9	168.5	172.9	176.4
		312	141.6	144.2	147.9	153.1	154.3	158.6	161.5
		313	155.1	158.1	159.9	166.4	167.3	168.8	173.6
		314	155.4	159.1	159.2	166.7	169.1	171.1	175.8
		315	147.4	149.6	151.2	153.3	160.2	160.5	163.7
		316	148.9	149.7	150.4	157.1	157.1	159.4	164.1
		317	157.5	158.8	160.7	168.5	166.7	171.3	176.9
		318	160.5	164.6	163.1	173.8	170.0	180.1	182.6
		319	159.9	163.8	164.6	169.6	170.6	172.2	172.0
		320	152.3	155.3	154.3	161.4	164.5	165.3	168.6
		321	167.4	174.4	175.2	181.3	158.6	187.7	189.1
		322	162.0	169.0	172.1	175.0	152.8	179.6	185.9
		323	154.8	156.2	158.2	160.5	139.8	165.3	167.5
		324	162.0	166.4	170.5	171.9	153.4	177.9	180.7
		325	146.8	153.1	155.9	159.8	139.6	164.7	169.6
		326	160.4	164.3	167.1	170.4	173.8	177.7	179.5
		327	164.7	167.7	169.6	177.5	176.7	181.1	185.2
		328	143.9	147.6	150.5	153.3	153.7	159.0	161.3
		329	158.6	163.0	166.0	169.6	172.5	180.0	180.7

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
5	f	330	158.5	165.8	166.7	173.9	174.4	179.1	181.1
		331	163.1	168.1	172.8	176.3	182.2	184.0	186.2
		332	152.4	155.0	156.8	162.8	164.4	167.3	174.0
		333	140.8	142.3	147.8	148.2	149.5	152.1	157.7
		334	151.8	155.2	159.8	161.8	162.4	163.9	169.0
		335	152.2	153.8	160.2	162.1	164.3	167.9	171.3
		336	166.8	169.1	173.0	180.0	180.3	182.4	187.2
		337	164.0	166.2	166.3	175.1	172.5	175.9	178.8
		338	143.3	146.3	148.8	153.0	152.8	155.5	158.3
		339	152.9	154.5	154.7	157.7	160.4	163.6	166.8
		340	161.6	165.5	164.0	172.1	169.3	173.7	176.5
		341	157.6	161.4	158.6	165.6	162.5	167.5	171.5
		342	158.7	163.1	164.6	169.2	167.9	171.9	180.5
		343	164.8	172.5	169.9	175.7	175.3	179.6	182.5
		344	163.3	174.8	171.6	175.5	178.3	176.0	182.0
		345	149.8	155.1	153.6	158.2	156.7	159.9	163.1
		346	154.8	158.3	165.6	166.7	170.6	176.6	177.1
		347	155.9	157.9	161.4	163.7	169.7	170.5	170.6
		348	160.7	162.9	169.4	170.0	173.7	177.6	180.2
		349	151.8	158.5	159.9	159.9	165.6	165.3	170.5
		350	155.3	157.6	162.4	163.7	170.3	170.5	174.6
		351	158.2	156.7	163.2	162.8	162.2	169.3	168.4
		352	149.3	148.4	152.3	157.6	158.7	163.2	164.8
		353	150.0	148.5	152.3	155.6	155.5	161.4	161.5
		354	163.8	163.0	170.3	162.7	168.4	178.0	180.7
		355	153.1	158.5	162.3	163.2	159.8	162.8	167.4
		356	150.6	150.0	155.3	156.1	158.6	162.1	164.0
		357	155.9	159.4	162.3	165.1	167.2	168.3	176.3
		358	165.2	168.4	172.0	175.7	175.2	177.2	182.6

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
5	f	359	160.7	159.3	161.9	168.1	168.1	172.8	171.7
		360	156.6	155.9	160.7	164.0	167.6	171.7	175.1
		361	.	.	.	.	.	.	.
		362	.	.	.	.	.	.	.
		363	.	.	.	.	.	.	.
		364	.	.	.	.	.	.	.
		365	.	.	.	.	.	.	.
		366	.	.	.	.	.	.	.
		367	.	.	.	.	.	.	.
		368	.	.	.	.	.	.	.
		369	.	.	.	.	.	.	.
		370	.	.	.	.	.	.	.
		371	.	.	.	.	.	.	.
		372	.	.	.	.	.	.	.
		373	.	.	.	.	.	.	.
		374	.	.	.	.	.	.	.
		375	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
6	f	376	.	.	.	.	.	.	.
		377	.	.	.	.	.	.	.
		378	.	.	.	.	.	.	.
		379	.	.	.	.	.	.	.
		380	.	.	.	.	.	.	.
		381	.	.	.	.	.	.	.
		382	.	.	.	.	.	.	.
		383	.	.	.	.	.	.	.
		384	.	.	.	.	.	.	.
		385	.	.	.	.	.	.	.
		386	154.0	153.4	156.1	162.4	162.7	165.5	170.1
		387	152.8	155.2	157.2	161.4	160.7	164.0	169.1
		388	143.7	145.3	148.3	155.0	155.0	158.8	160.6
		389	143.4	145.2	146.5	151.5	153.0	154.5	160.5
		390	158.0	158.8	162.7	167.6	165.1	169.8	172.5
		391	153.5	155.3	157.0	160.0	160.2	163.3	166.5
		392	155.0	157.6	159.2	167.4	165.9	166.6	169.6
		393	140.0	142.3	145.3	148.9	150.1	150.5	154.8
		394	149.8	152.8	152.3	160.6	158.9	160.8	162.7
		395	154.6	157.4	162.4	168.4	164.0	167.9	168.6
		396	152.3	155.0	159.7	164.5	161.8	169.4	171.1
		397	168.3	170.4	175.8	178.6	180.7	182.6	183.0
		398	152.8	156.8	160.5	164.4	164.1	169.4	171.5
		399	155.2	161.4	162.8	168.1	169.4	172.3	176.5
		400	163.1	170.3	167.8	178.2	178.4	181.0	183.4
		401	146.3	153.2	154.0	156.4	157.8	162.6	163.6
		402	156.3	161.9	161.8	168.5	170.0	171.6	173.1
		403	156.5	162.3	161.7	168.8	169.1	173.5	176.4
		404	152.4	156.3	161.4	161.2	163.9	166.7	166.7

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
6	f	405	182.2	187.4	188.1	192.0	194.4	202.3	201.6
		406	145.1	142.9	150.6	149.2	149.7	153.8	158.0
		407	157.7	160.6	163.8	167.6	169.1	173.7	175.6
		408	160.9	162.5	169.3	168.7	177.1	178.1	181.1
		409	145.2	145.2	149.2	153.1	153.7	155.2	160.7
		410	156.8	157.7	159.3	165.0	169.4	168.7	177.2
		411	158.5	166.1	167.1	169.8	174.0	171.1	176.4
		412	141.2	143.9	145.0	147.7	151.0	150.6	151.8
		413	157.5	161.2	160.8	168.9	169.9	170.6	175.4
		414	151.9	156.4	155.4	159.4	162.6	161.9	166.1
		415	153.3	153.5	156.7	161.8	160.9	163.8	164.2
		416	157.4	165.8	164.9	169.4	174.8	176.1	177.7
		417	165.9	174.9	174.3	186.1	187.1	191.3	190.6
		418	158.1	165.9	161.1	161.8	165.1	165.7	168.2
		419	148.6	151.3	151.2	158.3	156.2	160.0	164.5
		420	152.6	162.6	160.0	168.3	167.9	168.7	175.5
		421	159.0	160.0	165.6	165.3	167.5	171.1	172.7
		422	153.6	153.8	157.9	162.9	166.0	167.7	169.8
		423	161.2	159.7	167.7	168.1	160.5	172.6	174.8
		424	155.5	156.0	163.8	164.0	168.3	169.1	170.8
		425	149.2	150.4	156.8	158.6	160.5	165.3	166.0
		426	154.9	161.3	162.3	164.7	164.4	166.0	170.0
		427	155.1	158.1	164.3	166.8	167.3	172.7	175.4
		428	154.8	156.7	162.1	165.1	165.2	164.7	168.9
		429	152.3	151.8	158.2	161.0	159.6	169.6	174.3
		430	163.7	164.2	167.3	169.2	171.1	175.8	181.0
		431	142.2	149.7	153.0	154.0	161.0	160.9	166.8
		432	154.5	158.3	166.3	168.5	169.7	175.5	180.8
		433	153.8	162.9	161.2	166.9	168.4	172.7	178.2

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Body Weights (grams)

			Day numbers relative to Start Date						
Group	Sex	Animal	50	57	64	71	78	85	91
6	f	434	152.0	160.5	160.3	169.1	170.4	173.2	178.9
		435	155.3	163.6	169.1	171.1	177.6	179.7	183.4
		436	.	.	.	.	.	.	.
		437	.	.	.	.	.	.	.
		438	.	.	.	.	.	.	.
		439	.	.	.	.	.	.	.
		440	.	.	.	.	.	.	.
		441	.	.	.	.	.	.	.
		442	.	.	.	.	.	.	.
		443	.	.	.	.	.	.	.
		444	.	.	.	.	.	.	.
		445	.	.	.	.	.	.	.
		446	.	.	.	.	.	.	.
		447	.	.	.	.	.	.	.
		448	.	.	.	.	.	.	.
		449	.	.	.	.	.	.	.
		450	.	.	.	.	.	.	.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Appendix E

Individual Food Consumption

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 76	71 / 77	71 / 78	76 / 78
1	f	1	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		2	5		11*	.	.	.	.	.	.	.	.	.	.	.	.	.
		3	5		11	10	12	9	11	11	7	11	10	10	.	.	10	.
		4	5		9	12	10	10	11	10	10	10	10	10	.	.	10	.
		5	5		9	8*	11	13	11	10	9	10	10	10	.	.	10	.
		6	5		9	8*	12	10	10	10	10	10	10	10	.	.	10	.
		7	5		10	12	11	10	10	10	10	9	10	10	.	.	9	.
		8	5		9*	12	10	10	10	10	9	10	9	9	.	.	9	.
		9	5		11*	11	11	10	8	10	10	10	10	9	.	.	9	.
		10	5		9	11	11	11	9	10	9	10	9	9	.	.	9	.
		11	5		11	11	11	11	10	11	10	10	11	10*	.	10	.	.
		12	5		11	12	12	13*	11	11	11	11	10	10	10	.	.	11
		13	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.
		14	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.
		15	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	77 / 78	78 / 85	85 / 91
1	f	1	5		.	.	.
		2	5		.	.	.
		3	5		.	10	10
		4	5		.	10	10
		5	5		.	9	10
		6	5		.	10	10
		7	5		.	9	10
		8	5		.	9	10
		9	5		.	9	10
		10	5		.	9	10
		11	5		10	10	11
		12	5		.	11	10
		13	5		.	.	.
		14	5		.	.	.
		15	5		.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 76	71 / 77	71 / 78	76 / 78
2	f	16	5		11*	.	.	.	.	.	.	.	.	.	.	.	.	.
		17	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		18	5		.	10	13	11	10	11	10	11	11	10	.	.	10	.
		19	5		10	12	10	10	10	10	10	10	10	10	.	.	9	.
		20	5		9	9*	12	10	10	10	10	10	10	10	.	.	10	.
		21	5		10	8	12	13	11	10	10	10	10	10	.	.	9	.
		22	5		9	11	10	10	10	10	9	9	10	9	.	.	9	.
		23	5		11	11*	11	10	10	10	10	10	10	9	.	.	9	.
		24	5		9*	13*	10	10	10	10	10	8	10	10	.	.	10	.
		25	5		9	12	10	11	10	11	10	10	9	10	.	.	9	.
		26	5		11	11	11	10	10	11	10	10	11	11	.	9	.	.
		27	5		11	11	11	10	10	10	10	10	9	9	11	.	.	10
		28	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		29	5		12	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		30	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	77 / 78	78 / 85	85 / 91
2	f	16	5		.	.	.
		17	5		.	.	.
		18	5		.	10	11
		19	5		.	9	9
		20	5		.	10	10
		21	5		.	10	10
		22	5		.	9	9
		23	5		.	9	10
		24	5		.	11	11
		25	5		.	10	9
		26	5		12	10	10
		27	5		.	10	10
		28	5		.	.	.
		29	5		.	.	.
		30	5		.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 76	71 / 77	71 / 78	76 / 78
3	f	31	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		32	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		33	5		.	8	11	21*	17*	10	10	10	11	10	.	.	10	.
		34	5		9	12	10	10	10	10	10	10	10	9	.	.	10	.
		35	5		9	7	12	12	10	11	10	10	10	10	.	.	10	.
		36	5		9	8	12	10	10	10	10	10	10	11	.	.	10	.
		37	5		9	11	10	10	10	8	9	9	10	9	.	.	9	.
		38	5		9*	12*	10	10	10	9	9	10	9	10	.	.	9	.
		39	5		10	11	10	11	11	10	10	10	10	10	.	.	9	.
		40	5		10	12	10	10	10	9	9	10	9	9	.	.	9	.
		41	5		11	11	11	10	11	11	10	10	10	10	.	9	.	.
		42	5		11	11	11	14*	11	10	10	10	10	10	10	.	.	11
		43	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		44	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.	.
		45	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	77 / 78	78 / 85	85 / 91
3	f	31	5		.	.	.
		32	5		.	.	.
		33	5		.	10	7
		34	5		.	10	10
		35	5		.	9	10
		36	5		.	10	7
		37	5		.	9	10
		38	5		.	9	10
		39	5		.	10	9
		40	5		.	9	9
		41	5		9	10	11
		42	5		.	11	11
		43	5		.	.	.
		44	5		.	.	.
		45	5		.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: To:	1 8	8 15	15 22	22 29	29 36	36 43	43 50	50 57	57 64	64 71	71 76	71 77	71 78	76 78
4	f	46	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		47	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		48	5		.	.*	10	10	10	11	10	10	14*	11	.	.	9	.
		49	5		.	11	10	10	10	10	10	11	10	10	.	.	10	.
		50	5		8	8	13	10	10	14	10	10	10	11	.	.	10	.
		51	5		9	12	10	12	11	10	10	11	10	11	.	.	10	.
		52	5		9	11	10	10	10	10	9	9	9	9	.	.	9	.
		53	5		10*	12	10	11	10	10	11	9	10	9	.	.	9	.
		54	5		9*	12*	8	10	11	10	9	10	10	9	.	.	9	.
		55	5		11	10	11	10	10	10	10	10	9	9	.	.	10	.
		56	5		11	11	9	10	10	10	10	10	10	14*	.	10	.	.
		57	5		12	11	10	10	10	10	10	10	10	10	10	.	.	11
		58	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.
		59	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.
		60	5		11	.	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	77 / 78	78 / 85	85 / 91
4	f	46	5		.	.	.
		47	5		.	.	.
		48	5		.	10	9
		49	5		.	10	10
		50	5		.	9	10
		51	5		.	10	11
		52	5		.	9	9
		53	5		.	10	10*
		54	5		.	10	9
		55	5		.	10	12
		56	5		11	10	15*
		57	5		.	10	17*
		58	5		.	.	.
		59	5		.	.	.
		60	5		.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: To:	1 8	8 15	15 22	22 29	29 36	36 43	43 50	50 57	57 64	64 71	71 76	71 77	71 78	76 78
5	f	61	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		62	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		63	5		10	11	10	10	10	10	10	10	10	10	.	.	10	.
		64	5		.	10	12	10	10	10	10	10	10	10	.	.	9	.
		65	5		9	6*	11	14	11	11	11	9	11	10	.	.	14*	.
		66	5		9	9	12	11	11	10	10	10	10	11	.	.	10	.
		67	5		10	11	10	10	10	9	9	9	1*	9	.	.	9	.
		68	5		10*	12*	10	9	10	9	9	9	10	9	.	.	9	.
		69	5		10	11	11	11	11	10	10	10	10	10	.	.	9	.
		70	5		8	12	10	10	10	9	9	10	9	9	.	.	10	.
		71	5		10	11	8	10	10	10	10	10	9	10	.	10	.	.
		72	5		10	11	11	10	10	10	10	10	10	9	10	.	.	10
		73	5		10	.	.	.	.	.	.	.	.	.	.	.	.	.
		74	5		10	.	.	.	.	.	.	.	.	.	.	.	.	.
		75	5		8	.	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	77 / 78	78 / 85	85 / 91
5	f	61	5		.	.	.
		62	5		.	.	.
		63	5		.	10	10
		64	5		.	10	9
		65	5		.	12*	11
		66	5		.	10	10
		67	5		.	10	12*
		68	5		.	9	9
		69	5		.	10	9
		70	5		.	9	10
		71	5		10	10	10
		72	5		.	10	10
		73	5		.	.	.
		74	5		.	.	.
		75	5		.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: To:	1 8	8 15	15 22	22 29	29 36	36 43	43 50	50 57	57 64	64 71	71 76	71 77	71 78	76 78
6	f	76	5		7*	.	.	.	.	.	.	.	.	.	.	.	.	.
		77	5		10*	.	.	.	.	.	.	.	.	.	.	.	.	.
		78	5		.	.	11	12	10	10	10	10	10	10	.	.	10	.
		79	5		8	12	14*	10	10	10	10	10	10	10	.	.	11*	.
		80	5		10	11*	14	11	17*	11	11	10	11	11	.	.	10	.
		81	5		9	12	11	11	11	10	11	13*	11	11	.	.	10	.
		82	5		10	11	10	11	10	10	10	10	10	10	.	.	9	.
		83	5		10	10	10	10	10	10	10	10	11	12*	.	.	10	.
		84	5		9*	11	10	10	10	10	15*	11	12	10	.	.	11	.
		85	5		10	12	11	11	14	11	11	10	11	10	.	.	10	.
		86	5		10	11	16	11	12	11	10	10	11	12*	.	10	.	.
		87	5		10	11	11	11	11	10	10	10	10	10	10	.	.	10
		88	5		14*	.	.	.	.	.	.	.	.	.	.	.	.	.
		89	5		10	.	.	.	.	.	.	.	.	.	.	.	.	.
		90	5		7	.	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	77 / 78	78 / 85	85 / 91
6	f	76	5		.	.	.
		77	5		.	.	.
		78	5		.	8	9
		79	5		.	10	12*
		80	5		.	10	11
		81	5		.	13*	10
		82	5		.	9	10
		83	5		.	9	14*
		84	5		.	11	11
		85	5		.	9	10
		86	5		11	11	11
		87	5		.	11	11
		88	5		.	.	.
		89	5		.	.	.
		90	5		.	.	.

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Group	Sex	Cage	Day	Comments and Markers: Type	Marker	Comment
1	f	1	7	Remaining Result		Feeder empty not spilled
		2	7	Remaining Result		Feeder empty not spilled
		5	15	Remaining Result		Feeder empty not spilled
		6	15	Remaining Result		Feeder empty not spilled
		8	8	Remaining Result		Feeder empty not spilled
		9	8	Remaining Result		Feeder empty not spilled
		11	71	Remaining I/E/S	E	
				Remaining Result		Food spilled
		12	29	Remaining I/E/S	E	
				Remaining Result		Food spilled
2	f	16	7	Remaining Result		Feeder empty not spilled
		17	7	Remaining Result		Feeder empty not spilled
		20	15	Remaining Result		Feeder empty not spilled
		23	13	Remaining Result		Feeder empty not spilled
		24	8	Remaining Result		Feeder empty not spilled
			13	Remaining Result		Feeder empty not spilled
3	f	31	7	Remaining Result		Feeder empty not spilled

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Group	Sex	Cage	Day	Comments and Markers: Type	Marker	Comment
3	f	32	7	Remaining Result		Feeder empty not spilled
		33	23	Remaining I/E/S	E	
				Remaining Result		Food spilled
			34	Remaining I/E/S	E	
				Remaining Result		Food spilled
		38	8	Remaining Result		Feeder empty not spilled
			13	Remaining Result		Feeder empty not spilled
		42	29	Remaining I/E/S	E	
				Remaining Result		Food spilled
4	f	46	7	Remaining Result		Feeder empty not spilled
		47	7	Remaining Result		Feeder empty not spilled
		48	13	Remaining Result		Feeder empty not spilled
			60	Remaining I/E/S	E	
				Remaining Result		Food spilled
		53	8	Remaining Result		Feeder empty not spilled
			91	Remaining I/E/S	E	
				Remaining Result		Food spilled
		54	8	Remaining Result		Feeder empty not spilled
			13	Remaining Result		Feeder empty not spilled
		56	71	Remaining I/E/S	E	
				Remaining Result		Food spilled

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Group	Sex	Cage	Day	Comments and Markers: Type	Marker	Comment
4	f	56	91	Remaining I/E/S	E	
				Remaining Result		Food spilled
		57	91	Remaining I/E/S	E	
				Remaining Result		Food spilled
5	f	61	7	Remaining Result		Feeder empty not spilled
		62	7	Remaining Result		Feeder empty not spilled
		65	15	Remaining Result		Feeder empty not spilled
			74	Remaining I/E/S	E	
				Remaining Result		Food spilled
			85	Remaining I/E/S	E	
				Remaining Result		Food spilled
		67	91	Remaining I/E/S	E	
				Remaining Result		Food spilled
			61	Remaining I/E/S	E	
				Remaining Result		Food contaminated
			64	Remaining I/E/S	E	
				Remaining Result		feed was in feeder but animals were not able to access it.
		68	8	Remaining Result		Feeder empty not spilled
			13	Remaining Result		Feeder empty not spilled
6	f	76	7	Remaining Result		Feeder empty not spilled
			8	Remaining I/E/S	E	
				Remaining Result		Food spilled

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Food Consumption by Cage (grams/animal/day)

Group	Sex	Cage	Day	Comments and Markers: Type	Marker	Comment
6	f	77	7	Remaining Result		Feeder empty not spilled
		79	74	Remaining I/E/S	E	
				Remaining Result		Food spilled
			22	Remaining I/E/S	E	
				Remaining Result		Food spilled
			86	Remaining Result		Food spilled
		80	15	Remaining Result		Feeder empty not spilled
			32	Remaining I/E/S	E	
				Remaining Result		Food spilled
		81	85	Remaining I/E/S	E	
				Remaining Result		Food spilled
			53	Remaining I/E/S	E	
				Remaining Result		Food spilled
		83	71	Remaining I/E/S	E	
				Remaining Result		Food spilled
			91	Remaining I/E/S	E	
				Remaining Result		Food spilled
			88	Remaining I/E/S	E	
				Remaining Result		Food spilled
		84	8	Remaining Result		Feeder empty not spilled
			50	Remaining I/E/S	E	
				Remaining Result		Food spilled
		86	71	Remaining I/E/S	E	
				Remaining Result		Food spilled
		88	8	Remaining I/E/S	E	
				Remaining Result		Food spilled

* = Result to left has an associated comment or marker.
Marker = E implies value excluded from means.
Food consumption units are g/animal/day.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Appendix F

Individual Water Consumption

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 78	78 / 85	85 / 91
1	f	1	5		24	.	.	.	.	.	.	.	.	.	.	.	.
		2	5		25	.	.	.	.	.	.	.	.	.	.	.	.
		3	5		49*	22	25	40	24	23	33	27	24	21	21	20	20
		4	5		24	28	24	23	24	30	23	23	24	22	21	21	22
		5	5		24	19	24	23	25	23	22	22	23	23	22	21	21
		6	5		24	20	27	24	25	22	21	22	23	22	22	25*	21
		7	5		23	25	23	22	22	20	20	21	20	20	19	19	20
		8	5		24	26	23	22	22	21	20	21	20	19	20	21	20
		9	5		22	24	23	23	23	22	20	21	21	18	19	20	20
		10	5		22	42	27	26	25	26	24	24	22	23	21	21	21
		11	5		24	25	24	24	24	26	22	20	20	21	22	23	21
		12	5		26	25	25	25	24	24	23	23	20	22	22	23	21
		13	5		23	.	.	.	.	.	.	.	.	.	.	.	.
		14	5		25	.	.	.	.	.	.	.	.	.	.	.	.
		15	5		20	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 78	78 / 85	85 / 91
2	f	16	5		25	.	.	.	.	.	.	.	.	.	.	.	.
		17	5		23	.	.	.	.	.	.	.	.	.	.	.	.
		18	5		27	24	23	23	23	23	23	23	24	23	22	20	20
		19	5		24	26	24	23	22	22	22	24	23	22	20	19	21
		20	5		24	23	26	24	23	23	23	23	24	24	23	23	22
		21	5		23	19	26	25	24	23	23	22	22	21	19	22	21
		22	5		23	21	22	20	26	21	19	19	20	19	18	18	18
		23	5		27	26	24	23	22	23	20	23	23	22	21	21	21
		24	5		23	26	23	22	22	23	22	36	23	22	22	22	36
		25	5		21	24	22	22	21	21	20	20	20	19	19	19	19
		26	5		25	24	23	22	23	22	21	20	18	21	17	20	20
		27	5		26	25	23	23	24	23	23	32*	21	22	22	23	20
		28	5		25	.	.	.	.	.	.	.	.	.	.	.	.
		29	5		26	.	.	.	.	.	.	.	.	.	.	.	.
		30	5		26	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 78	78 / 85	85 / 91
3	f	31	5		23	.	.	.	.	.	.	.	.	.	.	.	.
		32	5		23	.	.	.	.	.	.	.	.	.	.	.	.
		33	5		25	16	27	27	26	25	23	24	25	26	21	23	12*
		34	5		25	26	23	22	23	22	21	23	22	22	21	21	20
		35	5		23	20	25	24	23	21	22	24	24	24	23	22	20
		36	5		21	21	24	23	23	23	21	25	22	21	22	22	21
		37	5		22	24	38*	21	22	22	19	21	22	22	19	21	20
		38	5		22	25	27	32	23	22	21	21	21	20	19	20	20
		39	5		21	24	24	23	24	24	22	21	22	21	21	22	43*
		40	5		20	25	24	23	21	20	21	21	19	20	20	20	19
		41	5		26	26	27	26	25	24	25	24	23	23	43*	23	22
		42	5		26	25	24	23	24	24	22	22	20	22	22	23	23
		43	5		26	.	.	.	.	.	.	.	.	.	.	.	.
		44	5		25	.	.	.	.	.	.	.	.	.	.	.	.
		45	5		25	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: To:	1 8	8 15	15 22	22 29	29 36	36 43	43 50	50 57	57 64	64 71	71 78	78 85	85 91
4	f	46	5		21	.	.	.	.	.	.	.	.	.	.	.	.
		47	5		23	.	.	.	.	.	.	.	.	.	.	.	.
		48	5		27	23	22	22	23	21	20	20	21	21	19	19	18
		49	5		25	23	23	22	22	21	21	21	21	21	19	19	20
		50	5		21	21	24	22	22	19	20	20	20	19	19	19	18
		51	5		21	24	22	22	22	19	32*	20	20	20	19	20	18
		52	5		20	19	21	20	20	19	18	18	18	17	17	18	17
		53	5		24	24	36*	22	22	21	21	21	21	19	19	20	19
		54	5		22	25	21	20	21	20	20	20	19	19	18	19	18
		55	5		22	19	22	21	19	20	20	19	19	18	18	19	18
		56	5		24	24	22	21	21	21	21	19	20	20	23	20	18
		57	5		24	25	53	23	23	22	21	27*	20	21	20	20	19
		58	5		24	.	.	.	.	.	.	.	.	.	.	.	.
		59	5		27	.	.	.	.	.	.	.	.	.	.	.	.
		60	5		25	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 78	78 / 85	85 / 91
5	f	61	5		22	.	.	.	.	.	.	.	.	.	.	.	.
		62	5		20	.	.	.	.	.	.	.	.	.	.	.	.
		63	5		21	20	20	19	18	18	18	17	19	17	16	16	16
		64	5		21	20	19	17	18	18	17	17	17	16	16	17	16
		65	5		21	14	22	20	20	18	19	18	18	17	29*	22*	17
		66	5		19	15	21	20	19	18	18	18	18	17	17	17	16
		67	5		19	19	19	17	17	17	16	17	15	16	15	16	18
		68	5		20	19	18	17	17	17	17	16	17	16	16	16	15
		69	5		20	19	19	19	19	18	18	18	18	17	16	18	20
		70	5		18	21	19	18	17	17	17	16	17	15	16	16	15
		71	5		20	19	18	18	18	17	17	16	16	16	15	16	16
		72	5		23	22	20	20	19	20	18	18	17	17	16	19	17
		73	5		22	.	.	.	.	.	.	.	.	.	.	.	.
		74	5		23	.	.	.	.	.	.	.	.	.	.	.	.
		75	5		14	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Day numbers relative to Start Date

Group	Sex	Cage	No In Cage	From: / To:	1 / 8	8 / 15	15 / 22	22 / 29	29 / 36	36 / 43	43 / 50	50 / 57	57 / 64	64 / 71	71 / 78	78 / 85	85 / 91
6	f	76	5		18	.	.	.	.	.	.	.	.	.	.	.	.
		77	5		18	.	.	.	.	.	.	.	.	.	.	.	.
		78	5		17	15	17	16	25	16	16	15	16	15	14	12	15
		79	5		15	17	16	17	17	16	15	16	16	15	14	14	22
		80	5		18	15	19	18	18	17	17	16	16	16	36*	15	15
		81	5		14	19	17	16	17	16	16	16	16	28	14	14	14
		82	5		17	17	17	18	17	16	17	16	17	16	15	15	15
		83	5		17	16	17	16	16	16	15	15	16	15	17	15	13
		84	5		16	16	16	16	16	16	17	18	16	16	16	17	15
		85	5		17	15	16	26	16	15	16	16	16	15	14	14	14
		86	5		16	18	18	18	18	18	17	16	27	16	16	16	16
		87	5		18	19	19	18	24	18	31*	18	17	16	17	17	16
		88	5		17	.	.	.	.	.	.	.	.	.	.	.	.
		89	5		16	.	.	.	.	.	.	.	.	.	.	.	.
		90	5		32	.	.	.	.	.	.	.	.	.	.	.	.

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Water Consumption by Cage (mL/animal/day)

Group	Sex	Cage	Day	Comments and Markers: Type	Marker	Comment
1	f	3	1	Remaining I/E/S	E	
				Remaining Result		Water spilled
		6	79	Remaining I/E/S	E	
				Remaining Result		Water spilled
2	f	27	56	Remaining I/E/S	E	
				Remaining Result		Water spilled
3	f	33	91	Remaining Result		animals could not access water due to faulty sipper tube
		37	20	Remaining I/E/S	E	
				Remaining Result		Water spilled
		39	89	Remaining I/E/S	E	
				Remaining Result		Water spilled
		41	72	Remaining I/E/S	E	
				Remaining Result		Water spilled
4	f	51	48	Remaining I/E/S	E	
				Remaining Result		Water spilled
		53	19	Remaining I/E/S	E	
				Remaining Result		Water spilled
		57	53	Remaining I/E/S	E	
				Remaining Result		Water spilled
5	f	65	78	Remaining I/E/S	E	
				Remaining Result		Water spilled
6	f	80	73	Remaining I/E/S	E	
				Remaining Result		Water spilled
		87	50	Remaining I/E/S	E	
				Remaining Result		Water spilled

* = Result to left has an associated comment or marker.
Water consumption units are mL/animal/day.
Marker = E implies value excluded from means.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Appendix G

Immunology Contributing Scientist Report

Immunology Contributing Scientist Report for

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Submitted by:

Richard D. May, Ph.D.
Immunologist
Southern Research
Birmingham, Alabama

Southern Research Study Number: 13026.01.02

June 17, 2011

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1.0 SIGNATURE PAGE

Immunology Contributing Scientist Report for

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Southern Research Study Number: 13026.01.02

6/17/11

Richard D. May, Ph.D.
Immunologist
Southern Research

Date

2.0 INTRODUCTION

The objective of this aspect of the study was to examine Day 91 samples, as follows: (1) ferritin and transferrin serum levels from selected study animals by enzyme-linked immunosorbent assay (ELISA), (2) total 8-iso-prostaglandin F2α (8-isoprostane, or 8-iso) levels of oral cavity and duodenum homogenates from selected study animals by ELISA, (3) 8-hydroxydeoxyguanosine (8-OHdG) levels in the DNA extracted from oral cavity and duodenum tissues from selected study animals by ELISA, and (4) cytokine/chemokine levels in serum and oral cavity and duodenum homogenates by multiplexing.

3.0 METHODS AND MATERIALS

Homogenate Preparation of Oral Cavity and Duodenum Tissues: Protease Inhibitor Cocktail I [containing 4-(2-Aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF), EDTA, bestatin, E-64, leupeptin, and aprotinin] was obtained from Millipore Corporation (Billerica, MA). Tris, Tween 20, and NaCl were purchased from Sigma-Aldrich (St. Louis, MO). Phenylmethanesulfonyl fluoride (PMSF) was purchased from MP Biomedicals (Santa Ana, CA). 2-Propanol and NaOH were obtained from Fisher Scientific (Pittsburgh, PA). Homogenization buffer solution was prepared by weighing out PMSF and dissolving in 2-propanol. The solution was vortexed ~30 seconds and set aside. Tris was weighed and transferred to a beaker, to which deionized (DI) water was added. While stirring, the pH was adjusted to 7.5 with 1N NaOH. NaCl, Tween 20, and 1 mL of the PMSF/2-propanol solution were added to the Tris/DI water solution. Protease Inhibitor Cocktail I was reconstituted by adding 10 mL of DI water; it was then vortexed ~30 seconds, and added to the Tris/DI water/NaCl/Tween 20/PMSF solution. The final solution was stirred ~7 minutes to insure a uniform mixture. The final solution was clear. The final concentrations of the constituents in the homogenization buffer were 20 mmol/L Tris, 150 mmol/L NaCl, 1% 2-propanol, 0.174 mg/mL PMSF, 0.05% Tween-20, 2 mM AEBSF, 1 mM EDTA, 130 μM bestatin, 14 μM E-64, 1 μM leupeptin, and 0.3 μM aprotinin. Tissues were then homogenized in 0.3 mL of this buffer using an Omni THQ homogenizer (Omni International; Kennesaw, GA) and a disposable hard tissue tip. After homogenization, samples were frozen at -80 °C until the supernatants were assayed for free 8-isoprostane and various cytokines and chemokines, as described below.

DNA Extraction of Oral Cavity and Duodenum Tissues: Gentra PureGene Tissue Kits were purchased from Qiagen (Valencia, CA) and used to extract DNA from the tissues. 600 µL of cell lysis buffer (included in the Qiagen kit) were added to each tissue vial. The tissues were then homogenized using the Omni THQ homogenizer and a disposable hard tissue tip. DNA was extracted following the manufacturer's instructions. Genomic DNA samples were rehydrated with 50 and 100 µL of DNA hydration solution (included in the Qiagen kit) for the oral cavity and duodenum samples, respectively. DNA concentrations were then determined using the PicoGreen kit Invitrogen (Carlsbad, CA) and a Bio-Rad Versafluor fluorimeter (Hercules, CA). Extracted DNA was dissolved in water and converted to single-stranded DNA by incubating the samples at 95 ºC for 5 minutes and then chilling them on ice. After this, samples were digested to nucleosides by incubating the denatured DNA with 20 units of nuclease P1 (Sigma-Aldrich) for 2 hours at 37 ºC in 20 mM sodium acetate, pH 5.2, followed by treatment with 10 units of alkaline phosphatase (Sigma-Aldrich) for 1 hour at 37 ºC in 100 mM Tris, pH 7.5. Finally, the reaction mixture was centrifuged for 5 minutes at 6000 × g; samples were frozen at -80 °C until they were assayed for 8-OHdG, as described below.

Ferritin, Transferrin, 8-Isoprostane, and 8-OHdG ELISAs: Commercial ELISA kits to measure mouse ferritin and transferrin in serum were purchased from ALPCO (Salem, NH). Commercial ELISA kits to measure 8-isoprostane and 8-OHdG in oral cavity and duodenum homogenates were obtained from Cell Biolabs (San Diego, CA). These assays were performed according to the manufacturer's instructions; kit inserts are included in Attachment G1. For assays of 8-isoprostane, the hydrolysis step (samples are incubated at 45 ºC for 2 hours with 1 part 10 N NaOH for every 4 parts of liquid sample, followed by the addition of 100 µL of 10 N HCl per 500 µL of hydrolyzed sample) that is a part of the homogenate preparation was included. Thus, the assay measured total 8-isoprostane levels in this study.

It should be noted that the 8-OHdG ELISA assays were performed following conversion of the extracted DNA to single-stranded DNA, as called for by the kit instructions. Although the total amount of DNA in some reactions exceeded the maximum recommended in the kit inserts, a pilot study using samples containing high and low DNA concentrations demonstrated that this had no effect on the outcome of the assay.

Cytokine/Chemokine Analyses: A Milliplex kit was purchased from Millipore Corporation (Billerica, MA). Oral cavity and duodenum homogenates and sera were analyzed for the 23 cytokines/chemokines listed below on a Luminex 200 (Austin, TX). These assays were performed according to the manufacturer's instructions; kit inserts are included in Attachment G2.

Statistics: Statistics were performed using Provantis, utilizing ANOVA and the Dunnett's Test for significance.

Cytokines and Chemokines Analyzed in this Study

Complete Name	Abbreviation
Eosinophil chemotaxin (eotaxin)	Eotaxin
Granulocyte-colony stimulating factor	G-CSF
Granulocyte/macrophage-colony stimulating factor	GM-CSF
Interferon-gamma	IFN-γ
Interleukin-1-alpha	IL-1α
Interleukin-1-beta	IL-1β
Interleukins 2, 4, 5, 6, and 10	IL-2, IL-4, IL-5, IL-6, and IL-10
Interleukin 12 p70 subunit	IL-12 (p70)
Interleukins 13, 17, and 18	IL-13, IL-17, IL-18
Chemokine (C-X-C motif) ligand 10 or IFN-γ-induced protein 10	CXCL10 or IP-10
Chemokine (C-X-C motif) ligand 1	CXCL1, GROα, or KC
Leptin	Leptin
Monocyte chemotactic protein-1	MCP-1
Macrophage inflammatory protein-1-alpha	MIP-1α
Chemokine (C-C motif) ligand 5	CCL5 or RANTES
Tumor necrosis factor-alpha	TNF-α
Vascular endothelial growth factor	VEGF

4.0 RESULTS AND DISCUSSION

Iron Analysis (Ferritin and Transferrin Serum Levels): Serum samples prepared from blood taken on Day 91 were diluted with kit assay buffer at 1:40 for ferritin and at 1:40,000 for transferrin and were assayed in an effort to examine iron analysis following treatment with sodium dichromate dihydrate (SDD). The individual and summary data are presented in Table G1 and Table G2, respectively. The statistical analyses indicated that there were no differences among the groups in terms of circulating ferritin or transferrin levels.

8-Isoprostane Levels in Oral Cavity and Duodenum Homogenates: Homogenates of oral cavity and duodenum tissues taken on Day 91 were diluted with kit assay buffer and were analyzed for total 8-isoprostane in an effort to determine if SDD treatment caused any oxidative stress, as assessed by this eicosanoid. The individual and summary data are presented in Table G3 and Table G4, respectively. Although there was a statistically significant increase in 8-isoprostane in oral cavity for rats in the 60 mg/mL group, this was considered to be a spurious because of the lack of a dose response in the other groups. Thus, the data suggest that for oral cavity and duodenum there were no apparent inter-group differences in the levels of total 8-isoprostane.

8-OHdG Levels in Oral Cavity and Duodenum Tissues: DNA samples that had been extracted from oral cavity and duodenum tissues taken on Day 91 were diluted with kit assay buffer and were analyzed for 8-OHdG in an effort to determine if SDD treatment caused any oxidative DNA damage, as assessed by assaying for this marker of oxidative stress. The results from the assay were normalized to the amount of DNA in each sample; individual results are presented in Table G3 and summary results are presented in Table G4. There were no statistically or biologically significant changes in 8-OHdG levels in either oral cavity or duodenum.

Cytokine/Chemokine Levels in Serum, Oral Cavity, and Duodenum: Undiluted serum and homogenates of oral cavity and duodenum tissues taken on Day 91 were analyzed for their levels of 23 cytokines/chemokines by multiplexing. As shown in Table G5 (individual data) and Table G6 (summary data), many of the analytes were at low or background levels in the serum.

Those with notable levels were GRO/KC (the rat equivalent of human IL-8), IL-2, IL-6, IL-12p70, leptin, MCP-1, and RANTES. The results of the statistical analyses showed that IL-12p70 levels for rats in the 60 mg/L group and leptin levels for rats in the 520 mg/L group were lower than those in the control group. As shown in Table G7 (individual data) and Table G8 (summary data), most of the analytes were at low or background levels in the oral cavity homogenates. Those with notable levels were IL-1α, IL-18, leptin, and VEGF. However, there appeared to be no differences among the groups in terms of these or the other cytokines/chemokines in the oral cavity homogenates. As shown in Table G9 (individual data) and Table G10 (summary data), most of the analytes were at low or background levels in the duodenum homogenates. Those with notable levels were IL-1α, IL-1β, IL-18, leptin, RANTES, and VEGF. However, other than the results for IL-1α, there appeared to be no differences among the groups in terms of these or the other cytokines/chemokines in the duodenum homogenates. For the pro-inflammatory cytokine IL-1α, there was a statistically significant increase in the 170 and 520 mg/L groups in comparison with the vehicle control group. Moreover, the levels of IL-1α in duodenum homogenates appeared to be elevated, although not statistically so, for animals in the 60 mg/L group.

Protein Concentrations in Tissue Homogenates: The results of the protein assays performed on tissue homogenates used for assay of 8-isoprostane and cytokines/chemokines are presented in Table G11.

5.0 CONCLUSIONS

The objective of this phase of the study was to examine Day 91 samples for: (1) ferritin and transferrin serum levels to look at iron content, (2) total 8-isoprostane levels of oral cavity and duodenum homogenates of selected study animals by ELISA, (3) 8-OHdG levels of the DNA extracted from oral cavity and duodenum tissues, and (4) cytokine/chemokine levels in serum and oral cavity and duodenum homogenates. The major findings of this part of the study were:

1) Transferrin levels in serum did not appear to be altered from the vehicle control in any of the SDD treatment groups.

2) Ferritin levels in serum did not appear to be altered from the vehicle control in any of the SDD treatment groups.

3) Total 8-isoprostane levels in the oral cavity and duodenum homogenates did not appear to be altered from the vehicle control in any of the SDD treatment groups.

4) 8-OHdG levels in the oral cavity and duodenum homogenates did not appear to be altered from the vehicle control in any of the SDD treatment groups.

5) The only statistically significant changes in the 23 cytokines/chemokines analyzed included an increase in the inflammatory cytokine IL-1α in the duodenum of groups administered SDD at 170 or 520 mg/L, a decrease in leptin in the serum of the group administered SDD at 520 mg/L, and a decrease in IL-12p70 in the serum of the group administered SDD at 60 mg/L.

6.0 PARTICIPATING PERSONNEL

Parrish L. Payne, B.S.
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Nicolas W. Powers, M.S.
Richard D. May, Ph.D.

Table G1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Ferritin and Transferrin Levels

Day: 91 relative to Start Date

Group	Sex	Animal	Ferritin Levels ng/mL	Transferrin Levels mg/ml
1	f	51	907.00	3.09
		52	1254.00	3.33
		53	709.00	3.14
		54	710.00	3.37
		55	847.00	2.35
2	f	126	1590.00	3.57
		127	1910.00	3.42
		128	1730.00	3.64
		129	1419.00	3.00
		130	1085.00	2.92
3	f	201	1016.00	2.97
		202	1176.00	3.01
		203	892.00	2.36
		204	811.00	2.95
		205	1124.00	3.38

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Ferritin and Transferrin Levels

Day: 91 relative to Start Date

Group	Sex	Animal	Ferritin Levels ng/mL	Transferrin Levels mg/ml
4	f	276	1298.00	2.94
		277	1734.00	2.40
		278	1744.00	2.59
		279	1020.00	2.56
		280	1850.00	2.66
5	f	351	1294.00	2.18
		352	1535.00	2.90
		353	1768.00	2.92
		354	1370.00	2.98
		355	602.00	2.36
6	f	426	517.00	2.53
		427	1224.00	2.68
		428	1655.00	2.70
		429	1279.00	2.10
		430	1575.00	2.55

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G2

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Serum Ferritin and Transferrin Levels

Day: 91 relative to Start Date

Group	Sex		Ferritin Levels ng/mL	Transferrin Levels mg/ml
1	f	Mean	885.400	3.056
		S.D.	223.433	0.412
		N	5	5
2	f	Mean	1546.800	3.310
		S.D.	315.014	0.330
		N	5	5
3	f	Mean	1003.800	2.934
		S.D.	153.233	0.366
		N	5	5
4	f	Mean	1529.200	2.630
		S.D.	354.871	0.198
		N	5	5
5	f	Mean	1313.800	2.668
		S.D.	437.446	0.370
		N	5	5
6	f	Mean	1250.000	2.512
		S.D.	449.571	0.242
		N	5	5

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual 8-OHdG and 8-Isoprostane Analyses in Tissue Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	8-OHdG Oral Cav ng/mg DNA	8-OHdG Duodenum ng/mg DNA	8-Iso Oral Cav ng/mL	8-Iso Duodenum ng/mL
1	f	31	9	7	147.72	203.16
		32	5	15	11.60	602.41
		33	5	7	112.56	180.13
		34	6	9	75.48	40.86
		35	19	11	192.93	13.59
2	f	106	3	8	19.44	635.36
		107	16	4	6.15	179.65
		108	9	10	4.75	16.11
		109	6	13	29.75	149.52
		110	9	10	5.79	56.95
3	f	181	10	8	15.57	418.37
		182	5	8	8.23	341.24
		183	9	9	994.48	73.43
		184	6	7	267.65	277.92
		185	12	10	1381.31	238.52

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual 8-OHdG and 8-Isoprostane Analyses in Tissue Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	8-OHdG Oral Cav ng/mg DNA	8-OHdG Duodenum ng/mg DNA	8-Iso Oral Cav ng/mL	8-Iso Duodenum ng/mL
4	f	256	11	7	618.26	14.12
		257	1	7	979.91	91.53
		258	6	12	1329.68	98.10
		259	23	2	4.73	560.44
		260	7	8	1270.07	416.54
5	f	331	19	9	672.63	2053.31
		332	6	5	41.03	45.09
		333	6	5	35.99	676.96
		334	3	9	297.27	83.96
		335	8	7	305.03	705.13
6	f	406	11	6	80.77	7.15
		407	12	9	26.71	499.99
		408	6	7	114.16	4.54
		409	3	13	118.07	8.31
		410	5	12	40.48	27.25

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of 8-OHdG and 8-Isoprostane Analyses in Tissue Homogenates

Day: 91 relative to Start Date

			8-OHdG Oral Cav ng/mg DNA	8-OHdG Duodenum ng/mg DNA	8-Iso Oral Cav ng/mL	8-Iso Duodenum ng/mL
1	f	Mean	8.8	9.8	108.058	208.030
		S.D.	5.9	3.3	69.214	235.632
		N	5	5	5	5
2	f	Mean	8.6	9.0	13.176	207.518
		S.D.	4.8	3.3	11.055	248.242
		N	5	5	5	5
3	f	Mean	8.4	8.4	533.448	269.896
		S.D.	2.9	1.1	621.715	129.211
		N	5	5	5	5
4	f	Mean	9.6	7.2	840.530*	236.146
		S.D.	8.3	3.6	545.702	238.209
		N	5	5	5	5
5	f	Mean	8.4	7.0	270.390	712.890
		S.D.	6.2	2.0	260.417	812.340
		N	5	5	5	5
6	f	Mean	7.4	9.4	76.038	109.448
		S.D.	3.9	3.0	41.653	218.506
		N	5	5	5	5

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)
[a]Data for 8-isoprostane in oral cavity had unequal variances. The data were $\log_{10}$ transformed prior to statistical analysis.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex	Animal	G-CSF Serum pg/mL	GM-CSF Serum pg/mL	IFN-γ Serum pg/mL	IL-10 Serum pg/mL	IL-12p70 Serum pg/mL	IL-13 Serum pg/mL	IL-17 Serum pg/mL	IL-1ß Serum pg/mL
1	f	31	6.1	BDL	56.5	BDL	110.1	89.3	BDL	BDL
		32	BDL	BDL	63.9	BDL	46.5	69.4	BDL	5.0
		33	BDL	BDL	118.2	BDL	91.5	136.4	BDL	BDL
		34	BDL	BDL	78.2	BDL	158.8	95.0	BDL	18.3
		35	BDL	BDL	14.1	78.3	BDL	BDL	BDL	BDL
2	f	106	BDL	BDL	BDL	BDL	BDL	24.4	BDL	32.3
		107	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		108	BDL	BDL	14.1	BDL	BDL	BDL	BDL	BDL
		109	BDL	BDL	48.8	BDL	11.9	52.1	BDL	BDL
		110	BDL	BDL	32.6	BDL	BDL	BDL	BDL	BDL
3	f	181	BDL	BDL	14.1	BDL	BDL	BDL	BDL	BDL
		182	BDL	BDL	105.3	BDL	46.5	158.4	61.9	18.8
		183	BDL	BDL	32.6	BDL	91.5	83.2	6.5	13.9
		184	BDL	BDL	14.1	BDL	BDL	BDL	BDL	BDL
		185	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex	Animal	G-CSF Serum pg/mL	GM-CSF Serum pg/mL	IFN-γ Serum pg/mL	IL-10 Serum pg/mL	IL-12p70 Serum pg/mL	IL-13 Serum pg/mL	IL-17 Serum pg/mL	IL-1ß Serum pg/mL
4	f	256	BDL	BDL	32.6	BDL	11.9	24.4	BDL	BDL
		257	BDL	BDL	BDL	BDL	11.9	BDL	BDL	BDL
		258	BDL	BDL	32.6	BDL	46.5	BDL	BDL	BDL
		259	BDL	BDL	BDL	BDL	BDL	105.5	BDL	BDL
		260	BDL	BDL	48.8	BDL	11.9	24.4	BDL	24.8
5	f	331	BDL	BDL	32.6	BDL	BDL	76.6	BDL	49.2
		332	BDL	BDL	40.9	28.4	BDL	52.1	BDL	5.0
		333	BDL	BDL	32.6	BDL	BDL	24.4	BDL	20.5
		334	BDL	BDL	14.1	BDL	BDL	BDL	BDL	BDL
		335	BDL	BDL	14.1	BDL	BDL	BDL	BDL	10.6
6	f	406	BDL	BDL	14.1	BDL	BDL	24.4	BDL	BDL
		407	BDL	BDL	BDL	BDL	BDL	61.4	BDL	BDL
		408	BDL	BDL	32.6	BDL	BDL	24.4	BDL	66.4
		409	BDL	BDL	32.6	BDL	BDL	BDL	BDL	BDL
		410	BDL	BDL	14.1	BDL	BDL	BDL	BDL	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex	Animal	IL-2 Serum pg/mL	IL-4 Serum pg/mL	IL-5 Serum pg/mL	IL-6 Serum pg/mL	IP-10 Serum pg/mL	MCP-1 Serum pg/mL	MIP-1α Serum pg/mL	RANTES Serum pg/mL
1	f	31	152.7	47.90	BDL	215.4	71.6	232.9	BDL	7877.1
		32	254.0	BDL	BDL	90.7	71.6	832.2	BDL	22934.9
		33	187.9	BDL	BDL	90.7	183.7	275.7	BDL	10629.8
		34	25.3	BDL	BDL	BDL	136.0	321.2	BDL	11262.1
		35	BDL	BDL	BDL	49.2	BDL	339.5	BDL	12269.5
2	f	106	326.1	BDL	BDL	49.2	BDL	326.5	BDL	25791.8
		107	BDL	BDL	BDL	90.7	BDL	371.2	BDL	20507.3
		108	43.2	BDL	BDL	49.2	BDL	243.4	BDL	10244.5
		109	164.6	47.90	BDL	128.6	BDL	304.9	BDL	13396.3
		110	59.2	BDL	BDL	90.7	BDL	275.7	BDL	12726.5
3	f	181	25.3	BDL	BDL	BDL	BDL	181.6	BDL	9329.9
		182	152.7	BDL	BDL	164.3	71.6	342.0	BDL	15541.0
		183	232.5	BDL	BDL	295.9	206.3	483.3	BDL	26206.0
		184	59.2	BDL	BDL	49.2	BDL	269.5	BDL	8825.1
		185	25.3	BDL	BDL	49.2	BDL	240.0	BDL	6678.6

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex	Animal	IL-2 Serum pg/mL	IL-4 Serum pg/mL	IL-5 Serum pg/mL	IL-6 Serum pg/mL	IP-10 Serum pg/mL	MCP-1 Serum pg/mL	MIP-1α Serum pg/mL	RANTES Serum pg/mL
4	f	256	88.4	BDL	BDL	90.7	BDL	415.8	BDL	29434.2
		257	BDL	BDL	BDL	49.2	BDL	243.4	BDL	9725.4
		258	115.2	47.90	BDL	90.7	BDL	250.1	BDL	8613.6
		259	140.5	BDL	BDL	49.2	BDL	281.7	BDL	12534.5
		260	254.0	BDL	BDL	49.2	BDL	448.7	BDL	52056.4
5	f	331	232.5	BDL	BDL	49.2	BDL	464.3	BDL	31946.8
		332	43.2	BDL	BDL	90.7	BDL	218.3	BDL	6374.8
		333	210.5	BDL	BDL	25.5	BDL	296.4	BDL	21974.6
		334	25.3	BDL	BDL	90.7	BDL	352.0	BDL	8805.4
		335	BDL	BDL	BDL	49.2	BDL	243.4	BDL	12094.1
6	f	406	140.5	BDL	BDL	70.6	BDL	366.5	BDL	23671.5
		407	BDL	BDL	BDL	25.5	BDL	336.9	BDL	5970.0
		408	88.4	BDL	BDL	90.7	BDL	315.9	BDL	19923.6
		409	115.2	BDL	BDL	BDL	BDL	329.2	BDL	21372.1
		410	254.0	BDL	BDL	70.6	BDL	407.2	BDL	17085.6

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex	Animal	TNF-α Serum pg/mL	IL-18 Serum pg/mL	Eotaxin Serum pg/mL	Leptin Serum pg/mL	VEGF Serum pg/mL	IL-1α Serum pg/mL	GRO/KC Serum pg/mL
1	f	31	32.6	46.3	BDL	27955.7	BDL	BDL	233.0
		32	19.3	38.4	BDL	25447.6	BDL	BDL	676.8
		33	41.0	54.2	BDL	23868.0	BDL	BDL	589.8
		34	27.2	30.4	BDL	18287.8	BDL	BDL	246.3
		35	BDL	42.4	BDL	21942.1	BDL	BDL	687.6
2	f	106	BDL	50.3	BDL	13492.5	BDL	BDL	625.1
		107	BDL	22.2	BDL	16344.1	BDL	BDL	505.0
		108	BDL	58.1	BDL	10656.5	BDL	BDL	207.9
		109	7.6	65.9	217.6	18728.9	BDL	BDL	356.8
		110	BDL	30.4	BDL	32184.8	BDL	BDL	381.5
3	f	181	BDL	7.4	BDL	17408.1	BDL	BDL	315.4
		182	BDL	54.2	BDL	16458.0	BDL	BDL	647.2
		183	9.0	48.3	194.8	20166.2	BDL	67.4	773.4
		184	BDL	30.4	BDL	19649.5	BDL	BDL	354.8
		185	BDL	30.4	BDL	19141.5	BDL	BDL	299.2

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G5

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex	Animal	TNF-α Serum pg/mL	IL-18 Serum pg/mL	Eotaxin Serum pg/mL	Leptin Serum pg/mL	VEGF Serum pg/mL	IL-1α Serum pg/mL	GRO/KC Serum pg/mL
4	f	256	BDL	30.4	BDL	26780.8	BDL	BDL	746.7
		257	BDL	30.4	BDL	21678.9	BDL	BDL	198.6
		258	BDL	38.4	BDL	18871.4	BDL	BDL	154.3
		259	15.0	38.4	BDL	19677.6	BDL	BDL	535.5
		260	BDL	42.4	BDL	14169.2	BDL	BDL	1194.9
5	f	331	BDL	50.3	BDL	28656.9	BDL	BDL	870.2
		332	BDL	34.4	BDL	21296.8	BDL	BDL	70.4
		333	BDL	30.4	BDL	11764.5	BDL	BDL	422.2
		334	BDL	34.4	BDL	13525.0	BDL	BDL	594.7
		335	BDL	13.9	BDL	15205.8	BDL	BDL	311.4
6	f	406	BDL	18.1	BDL	9767.2	BDL	BDL	841.7
		407	BDL	30.4	BDL	14705.6	BDL	BDL	411.3
		408	BDL	46.3	BDL	11465.6	BDL	BDL	198.6
		409	BDL	90.9	BDL	12117.8	BDL	BDL	838.8
		410	BDL	40.4	BDL	11969.1	BDL	BDL	472.2

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G6

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex		IFN-γ Serum pg/mL	IL-12p70 Serum pg/mL	IL-13 Serum pg/mL	IL-2 Serum pg/mL	IL-6 Serum pg/mL	IP-10 Serum pg/mL	MCP-1 Serum pg/mL	RANTES Serum pg/mL
1	f	Mean	66.18	101.73	97.53	154.98	111.50	115.73	400.30	12994.68
		S.D.	37.62	46.48	28.14	96.11	71.98	54.55	244.97	5790.47
		N	5	4	4	4	4	4	5	5
2	f	Mean	31.83	11.90	38.25	148.28	81.68	.	304.34	16533.28
		S.D.	17.36	.	.	130.21	33.44	.	48.72	6430.28
		N	3	1	2	4	5	0	5	5
3	f	Mean	41.53	69.00	120.80	99.00	139.65	138.95	303.28	13316.12
		S.D.	43.40	.	.	91.04	117.45	.	116.04	7924.88
		N	4	2	2	5	4	2	5	5
4	f	Mean	38.00	20.55*	51.43	149.53	65.80	.	327.94	22472.82
		S.D.	9.35	17.30	46.82	72.83	22.73	.	97.01	18553.93
		N	3	4	3	4	5	0	5	5
5	f	Mean	26.86	.	51.03	127.88	61.06	.	314.88	16239.14
		S.D.	12.13	.	26.12	108.73	28.74	.	98.09	10599.21
		N	5	0	3	4	5	0	5	5
6	f	Mean	23.35	.	36.73	149.53	64.35	.	351.14	17604.56
		S.D.	10.68	.	21.36	72.83	27.58	.	36.42	6928.43
		N	4	0	3	4	4	0	5	5

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G6

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex		IL-18 Serum pg/mL	Leptin Serum pg/mL	GRO/KC Serum pg/mL	G-CSF Serum pg/mL	GM-CSF Serum pg/mL	IL-10 Serum pg/mL	IL-17 Serum pg/mL	IL-1ß Serum pg/mL
1	f	Mean	42.34	23500.24	486.70	6.10	.	78.30	.	11.65
		S.D.	8.87	3652.94	228.74	.	.	.	.	.
		N	5	5	5	1	0	1	0	2
2	f	Mean	45.38	18281.36	415.26	.	.	.	.	32.30
		S.D.	18.50	8341.62	157.83	.	.	.	.	.
		N	5	5	5	0	0	0	0	1
3	f	Mean	34.14	18564.66	478.00	.	.	.	34.20	16.35
		S.D.	18.34	1569.25	217.64	.	.	.	.	.
		N	5	5	5	0	0	0	2	2
4	f	Mean	36.00	20235.58	566.00	.	.	.	.	24.80
		S.D.	5.37	4579.75	428.25	.	.	.	.	.
		N	5	5	5	0	0	0	0	1
5	f	Mean	32.68	18089.80	453.78	.	.	28.40	.	21.33
		S.D.	12.98	6912.18	300.63	.	.	.	.	19.66
		N	5	5	5	0	0	1	0	4
6	f	Mean	45.22	12005.06*	552.52	.	.	.	.	66.40
		S.D.	27.69	1775.24	281.62	.	.	.	.	.
		N	5	5	5	0	0	0	0	1

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G6

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Serum

Day: 91 relative to Start Date

Group	Sex		IL-4 Serum pg/mL	IL-5 Serum pg/mL	MIP-1α Serum pg/mL	TNF-α Serum pg/mL	Eotaxin Serum pg/mL	VEGF Serum pg/mL	IL-1α Serum pg/mL
1	f	Mean	47.900	.	.	30.03	.	.	.
		S.D.	.	.	.	9.13	.	.	.
		N	1	0	0	4	0	0	0
2	f	Mean	47.900	.	.	7.60	217.60	.	.
		S.D.	.	.	.	.	.	.	.
		N	1	0	0	1	1	0	0
3	f	Mean	.	.	.	9.00	194.80	.	67.40
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	1	1	0	1
4	f	Mean	47.900	.	.	15.00	.	.	.
		S.D.	.	.	.	.	.	.	.
		N	1	0	0	1	0	0	0
5	f	Mean	.	.	.	.	.	.	.
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	0	0	0	0
6	f	Mean	.	.	.	.	.	.	.
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	0	0	0	0

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	G-CSF Oral pg/mL	GM-CSF Oral pg/mL	IFN-γ Oral pg/mL	IL-10 Oral pg/mL	IL-12p70 Oral pg/mL	IL-13 Oral pg/mL	IL-17 Oral pg/mL	IL-1α Oral pg/mL
1	f	31	BDL	BDL	BDL	BDL	BDL	BDL	BDL	367.4
		32	BDL	BDL	BDL	41.7	BDL	BDL	BDL	360.9
		33	BDL	BDL	14.80	BDL	BDL	BDL	BDL	162.9
		34	BDL	BDL	BDL	BDL	BDL	BDL	BDL	260.7
		35	BDL	BDL	BDL	BDL	BDL	BDL	BDL	279.0
2	f	106	BDL	BDL	BDL	23.3	BDL	BDL	BDL	148.3
		107	BDL	BDL	BDL	BDL	BDL	BDL	BDL	523.4
		108	BDL	BDL	BDL	BDL	BDL	BDL	BDL	403.8
		109	BDL	BDL	BDL	BDL	BDL	BDL	BDL	297.2
		110	BDL	BDL	BDL	BDL	BDL	BDL	BDL	731.7
3	f	181	BDL	BDL	BDL	64.7	BDL	BDL	BDL	436.9
		182	BDL	BDL	BDL	BDL	BDL	BDL	BDL	91.2
		183	BDL	BDL	BDL	BDL	BDL	BDL	BDL	200.2
		184	BDL	BDL	BDL	BDL	BDL	BDL	BDL	434.0
		185	BDL	BDL	BDL	BDL	BDL	BDL	BDL	230.9

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	G-CSF Oral pg/mL	GM-CSF Oral pg/mL	IFN-γ Oral pg/mL	IL-10 Oral pg/mL	IL-12p70 Oral pg/mL	IL-13 Oral pg/mL	IL-17 Oral pg/mL	IL-1α Oral pg/mL
4	f	256	BDL	BDL	BDL	BDL	BDL	BDL	BDL	260.0
		257	BDL	BDL	BDL	BDL	BDL	BDL	BDL	173.6
		258	BDL	BDL	BDL	31.1	BDL	BDL	BDL	246.8
		259	BDL	BDL	BDL	9.6	BDL	BDL	BDL	290.2
		260	BDL	BDL	BDL	BDL	BDL	BDL	BDL	211.6
5	f	331	BDL	BDL	BDL	BDL	BDL	BDL	BDL	82.2
		332	BDL	BDL	BDL	BDL	BDL	BDL	BDL	204.3
		333	BDL	BDL	BDL	23.3	BDL	BDL	BDL	189.0
		334	BDL	BDL	BDL	BDL	BDL	BDL	BDL	382.8
		335	BDL	BDL	BDL	BDL	BDL	BDL	BDL	453.0
6	f	406	BDL	BDL	BDL	BDL	BDL	BDL	BDL	417.8
		407	BDL	BDL	BDL	BDL	BDL	BDL	BDL	476.2
		408	BDL	BDL	BDL	BDL	BDL	BDL	BDL	337.8
		409	BDL	BDL	BDL	40.0	BDL	BDL	BDL	288.5
		410	BDL	BDL	BDL	BDL	BDL	BDL	BDL	365.7

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	IL-1ß Oral pg/mL	IL-2 Oral pg/mL	IL-4 Oral pg/mL	IL-5 Oral pg/mL	IL-6 Oral pg/mL	IP-10 Oral pg/mL	MCP-1 Oral pg/mL	MIP-1α Oral pg/mL
1	f	31	44.3	11.2	BDL	BDL	BDL	BDL	4.9	BDL
		32	50.0	14.1	19.0	8.0	6.7	BDL	BDL	BDL
		33	24.7	11.2	BDL	BDL	6.7	18.4	7.4	BDL
		34	25.4	BDL	BDL	BDL	BDL	6.7	BDL	BDL
		35	34.2	11.2	7.7	9.3	6.7	BDL	7.4	BDL
2	f	106	18.7	11.2	BDL	BDL	BDL	BDL	BDL	BDL
		107	31.9	11.2	BDL	BDL	6.7	BDL	11.0	8.6
		108	30.7	11.2	BDL	4.9	BDL	BDL	BDL	BDL
		109	20.2	8.4	BDL	BDL	14.0	BDL	7.4	BDL
		110	38.6	5.6	6.0	BDL	BDL	BDL	BDL	BDL
3	f	181	22.4	14.1	BDL	BDL	6.7	9.7	11.0	BDL
		182	BDL	8.4	6.0	BDL	6.7	BDL	BDL	BDL
		183	15.4	12.7	BDL	BDL	BDL	BDL	7.4	BDL
		184	42.2	14.1	7.7	6.6	10.3	6.7	7.4	BDL
		185	30.1	5.6	BDL	BDL	BDL	BDL	7.4	8.2

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	IL-1ß Oral pg/mL	IL-2 Oral pg/mL	IL-4 Oral pg/mL	IL-5 Oral pg/mL	IL-6 Oral pg/mL	IP-10 Oral pg/mL	MCP-1 Oral pg/mL	MIP-1α Oral pg/mL
4	f	256	28.1	8.4	9.2	BDL	BDL	BDL	BDL	14.1
		257	20.3	5.6	BDL	BDL	6.7	6.7	BDL	BDL
		258	14.5	8.4	14.4	BDL	BDL	BDL	7.4	BDL
		259	24.3	11.2	BDL	BDL	BDL	BDL	7.4	BDL
		260	8.3	12.7	BDL	BDL	BDL	BDL	BDL	BDL
5	f	331	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		332	16.7	BDL	BDL	7.3	10.3	BDL	BDL	BDL
		333	22.1	BDL	BDL	BDL	10.3	BDL	7.4	BDL
		334	35.5	7.0	BDL	BDL	6.7	BDL	BDL	BDL
		335	35.7	8.4	BDL	BDL	6.7	9.7	BDL	BDL
6	f	406	45.8	11.2	BDL	BDL	6.7	BDL	BDL	BDL
		407	33.7	11.2	BDL	BDL	BDL	BDL	BDL	26.7
		408	39.4	8.4	BDL	BDL	BDL	BDL	BDL	BDL
		409	25.4	8.4	BDL	BDL	4.9	BDL	BDL	BDL
		410	33.3	8.4	7.7	BDL	10.3	BDL	BDL	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	RANTES Oral pg/mL	TNF-α Oral pg/mL	IL-18 Oral pg/mL	Eotaxin Oral pg/mL	Leptin Oral pg/mL	VEGF Oral pg/mL	GRO/KC Oral pg/mL
1	f	31	34.9	17.4	563.8	BDL	1409.2	508.7	48.5
		32	43.9	23.6	448.6	20.9	1056.9	153.5	37.0
		33	5.1	14.9	229.5	13.7	633.6	83.2	15.6
		34	18.5	7.6	181.5	BDL	613.4	255.2	9.0
		35	67.2	21.5	302.2	4.9	766.9	372.1	39.8
2	f	106	14.4	BDL	255.5	BDL	534.0	22.2	26.4
		107	92.8	20.0	415.8	BDL	1016.1	380.4	46.1
		108	22.9	17.0	343.6	23.5	716.6	399.7	45.8
		109	21.5	10.9	238.7	13.7	1036.5	850.7	12.4
		110	19.9	20.5	483.6	BDL	1315.8	654.5	43.4
3	f	181	121.8	6.8	216.1	23.5	781.1	297.8	38.4
		182	113.5	BDL	108.1	28.6	223.0	289.2	24.0
		183	72.3	BDL	146.3	40.9	679.0	504.6	15.6
		184	18.7	26.1	217.4	23.5	806.3	287.6	42.0
		185	25.1	22.4	159.6	13.7	796.9	209.4	38.4

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G7

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	RANTES Oral pg/mL	TNF-α Oral pg/mL	IL-18 Oral pg/mL	Eotaxin Oral pg/mL	Leptin Oral pg/mL	VEGF Oral pg/mL	GRO/KC Oral pg/mL
4	f	256	67.2	9.6	418.3	13.7	974.4	346.8	44.1
		257	138.8	BDL	163.3	BDL	618.5	187.7	24.0
		258	33.3	6.8	206.5	BDL	796.3	351.9	24.8
		259	104.6	BDL	273.2	17.8	987.2	232.6	48.2
		260	77.3	BDL	169.4	4.9	336.9	269.8	11.0
5	f	331	28.8	BDL	61.7	BDL	425.2	102.4	11.9
		332	48.8	6.8	220.6	20.9	630.0	181.8	7.9
		333	77.8	10.1	121.9	20.9	469.5	207.7	55.9
		334	91.2	BDL	516.5	BDL	600.9	240.8	52.2
		335	36.8	26.5	291.4	19.4	706.1	378.8	37.7
6	f	406	20.3	27.4	375.6	4.9	613.9	578.6	42.3
		407	34.8	25.8	532.1	4.9	912.6	656.9	47.9
		408	53.1	17.4	370.4	BDL	817.4	655.9	31.0
		409	9.5	18.5	176.6	17.8	502.5	233.7	15.6
		410	44.8	21.1	387.9	13.7	825.8	280.2	41.3

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 4.88 pg/mL).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G8

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex		IL-1α Oral pg/mL	IL-1ß Oral pg/mL	IL-2 Oral pg/mL	IL-6 Oral pg/mL	MCP-1 Oral pg/mL	RANTES Oral pg/mL	TNF-α Oral pg/mL	IL-18 Oral pg/mL
1	f	Mean	286.18	35.72	11.93	6.70	6.57	33.92	17.00	345.12
		S.D.	83.78	11.27	1.45	0.00	1.44	23.86	6.26	158.52
		N	5	5	4	3	3	5	5	5
2	f	Mean	420.88	28.02	9.52	10.35	9.20	34.30	17.10	347.44
		S.D.	221.93	8.40	2.50	.	.	32.86	4.41	104.29
		N	5	5	5	2	2	5	4	5
3	f	Mean	278.64	27.53	10.98	7.90	8.30	70.28	18.43	169.50
		S.D.	152.27	11.48	3.81	2.08	1.80	48.03	10.24	47.10
		N	5	4	5	3	4	5	3	5
4	f	Mean	236.44	19.10	9.26	6.70	7.40	84.24	8.20	246.14
		S.D.	45.05	7.86	2.76	.	.	39.76	.	105.70
		N	5	5	5	1	2	5	2	5
5	f	Mean	262.26	27.50	7.70	8.50	7.40	56.68	14.47	242.42
		S.D.	151.71	9.61	.	2.08	.	26.80	10.55	176.90
		N	5	4	2	4	1	5	3	5
6	f	Mean	377.20	35.52	9.52	7.30	.	32.50	22.04	368.52
		S.D.	72.46	7.61	1.53	2.75	.	17.74	4.41	126.51
		N	5	5	5	3	0	5	5	5

Statistics Test: One-way ANOVA run with no significance (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G8

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex		Eotaxin Oral pg/mL	Leptin Oral pg/mL	VEGF Oral pg/mL	GRO/KC Oral pg/mL	G-CSF Oral pg/mL	GM-CSF Oral pg/mL	IFN-γ Oral pg/mL	IL-10 Oral pg/mL
1	f	Mean	13.17	896.00	274.54	29.98	.	.	14.800	41.70
		S.D.	8.01	337.14	170.28	16.85	.	.	.	.
		N	3	5	5	5	0	0	1	1
2	f	Mean	18.60	923.80	461.50	34.82	.	.	.	23.30
		S.D.	.	304.04	313.09	14.96	.	.	.	.
		N	2	5	5	5	0	0	0	1
3	f	Mean	26.04	657.26	317.72	31.68	.	.	.	64.70
		S.D.	9.91	248.04	110.43	11.34	.	.	.	.
		N	5	5	5	5	0	0	0	1
4	f	Mean	12.13	742.66	277.76	30.42	.	.	.	20.35
		S.D.	6.59	272.23	71.55	15.44	.	.	.	.
		N	3	5	5	5	0	0	0	2
5	f	Mean	20.40	566.34	222.30	33.12	.	.	.	23.30
		S.D.	0.87	116.27	101.32	22.31	.	.	.	.
		N	3	5	5	5	0	0	0	1
6	f	Mean	10.33	734.44	481.06	35.62	.	.	.	40.00
		S.D.	6.48	169.78	207.69	12.75	.	.	.	.
		N	4	5	5	5	0	0	0	1

Statistics Test: One-way ANOVA run with no significance (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G8

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Oral Cavity Homogenates

Day: 91 relative to Start Date

Group	Sex		IL-12p70 Oral pg/mL	IL-13 Oral pg/mL	IL-17 Oral pg/mL	IL-4 Oral pg/mL	IL-5 Oral pg/mL	IP-10 Oral pg/mL	MIP-1α Oral pg/mL
1	f	Mean	.	.	.	13.35	8.65	12.55	.
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	2	2	2	0
2	f	Mean	.	.	.	6.00	4.90	.	8.60
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	1	1	0	1
3	f	Mean	.	.	.	6.85	6.60	8.20	8.20
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	2	1	2	1
4	f	Mean	.	.	.	11.80	.	6.70	14.10
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	2	0	1	1
5	f	Mean	.	.	.	.	7.30	9.70	.
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	0	1	1	0
6	f	Mean	.	.	.	7.70	.	.	26.70
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	1	0	0	1

Statistics Test: One-way ANOVA run with no significance (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G9

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	G-CSF Duodenum pg/mL	GM-CSF Duodenum pg/mL	IFN-γ Duodenum pg/mL	IL-10 Duodenum pg/mL	IL-12p70 Duodenum pg/mL	IL-13 Duodenum pg/mL	IL-17 Duodenum pg/mL	IL-1α Duodenum pg/mL
1	f	31	BDL	BDL	BDL	BDL	6.7	BDL	BDL	176.6
		32	BDL	BDL	81.1	158.3	BDL	BDL	BDL	319.3
		33	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		34	BDL	BDL	11.8	BDL	BDL	6.4	BDL	117.3
		35	BDL	BDL	BDL	BDL	BDL	BDL	BDL	309.0
2	f	106	BDL	BDL	6.8	BDL	9.8	BDL	BDL	302.4
		107	BDL	5.9	BDL	64.7	BDL	BDL	BDL	427.0
		108	BDL	BDL	BDL	BDL	6.7	BDL	BDL	342.3
		109	BDL	BDL	BDL	23.3	BDL	BDL	BDL	151.4
		110	BDL	12.6	BDL	BDL	24.2	6.4	BDL	199.8
3	f	181	BDL	BDL	11.8	169.6	BDL	BDL	BDL	405.2
		182	BDL	BDL	17.6	BDL	13.7	8.7	BDL	100.9
		183	BDL	7.8	BDL	172.4	BDL	6.4	BDL	281.8
		184	BDL	BDL	25.2	191.9	BDL	10.5	66.3	302.8
		185	BDL	BDL	4.9	BDL	9.8	6.4	BDL	168.3

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 24.41 pg/mL for Leptin, and 4.88 pg/mL for all other analytes).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G9

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	G-CSF Duodenum pg/mL	GM-CSF Duodenum pg/mL	IFN-γ Duodenum pg/mL	IL-10 Duodenum pg/mL	IL-12p70 Duodenum pg/mL	IL-13 Duodenum pg/mL	IL-17 Duodenum pg/mL	IL-1α Duodenum pg/mL
4	f	256	BDL	BDL	11.8	34.7	9.8	20.3	BDL	477.8
		257	BDL	BDL	BDL	27.3	BDL	BDL	BDL	373.7
		258	BDL	9.0	BDL	29.2	17.2	6.4	BDL	678.1
		259	BDL	7.2	4.9	51.8	8.3	13.5	BDL	587.0
		260	BDL	BDL	11.8	38.3	21.3	BDL	BDL	221.9
5	f	331	BDL	BDL	4.9	127.1	BDL	BDL	BDL	664.0
		332	BDL	BDL	11.8	BDL	12.5	BDL	BDL	800.5
		333	BDL	BDL	BDL	BDL	6.7	BDL	BDL	780.1
		334	BDL	BDL	11.8	BDL	BDL	BDL	BDL	228.4
		335	BDL	BDL	BDL	BDL	BDL	6.4	BDL	544.9
6	f	406	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		407	BDL	BDL	BDL	BDL	BDL	BDL	BDL	851.6
		408	BDL	BDL	BDL	BDL	BDL	BDL	BDL	523.8
		409	BDL	BDL	14.8	BDL	8.3	BDL	BDL	476.5
		410	BDL	BDL	BDL	BDL	BDL	BDL	BDL	758.1

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 24.41 pg/mL for Leptin, and 4.88 pg/mL for all other analytes).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G9

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	IL-1ß Duodenum pg/mL	IL-2 Duodenum pg/mL	IL-4 Duodenum pg/mL	IL-5 Duodenum pg/mL	IL-6 Duodenum pg/mL	IP-10 Duodenum pg/mL	MCP-1 Duodenum pg/mL	MIP-1α Duodenum pg/mL
1	f	31	16.7	5.6	10.4	BDL	17.8	BDL	19.5	BDL
		32	32.2	BDL	BDL	BDL	37.1	BDL	7.4	BDL
		33	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		34	97.8	34.0	14.4	9.8	10.3	6.7	32.2	5.1
		35	66.6	21.2	9.2	BDL	12.1	BDL	9.3	BDL
2	f	106	BDL	18.3	BDL	BDL	14.0	BDL	25.7	BDL
		107	18.0	8.4	BDL	BDL	23.5	BDL	BDL	BDL
		108	35.6	16.9	10.4	BDL	17.8	BDL	9.3	BDL
		109	196.3	14.1	12.6	4.9	BDL	BDL	42.4	5.0
		110	102.1	25.5	28.4	BDL	14.0	BDL	BDL	BDL
3	f	181	185.9	34.0	14.4	15.0	6.7	BDL	BDL	5.6
		182	33.1	36.9	12.6	BDL	25.4	6.7	18.4	5.0
		183	148.8	31.2	10.4	BDL	10.3	BDL	7.4	6.0
		184	77.9	48.3	17.6	34.5	6.7	BDL	BDL	BDL
		185	40.3	28.3	7.7	BDL	19.7	BDL	25.7	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 24.41 pg/mL for Leptin, and 4.88 pg/mL for all other analytes).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G9

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	IL-1ß Duodenum pg/mL	IL-2 Duodenum pg/mL	IL-4 Duodenum pg/mL	IL-5 Duodenum pg/mL	IL-6 Duodenum pg/mL	IP-10 Duodenum pg/mL	MCP-1 Duodenum pg/mL	MIP-1α Duodenum pg/mL
4	f	256	68.6	42.6	12.6	7.3	21.6	BDL	63.1	BDL
		257	150.0	8.4	7.7	BDL	14.0	BDL	37.9	BDL
		258	87.9	22.6	24.9	BDL	33.2	BDL	55.1	BDL
		259	151.7	24.0	14.4	8.0	14.0	BDL	55.1	5.1
		260	138.7	39.7	12.6	BDL	29.3	BDL	76.0	7.6
5	f	331	148.4	22.6	BDL	BDL	29.3	14.4	BDL	7.0
		332	21.2	22.6	6.0	BDL	BDL	BDL	74.6	BDL
		333	70.0	48.3	10.4	BDL	6.7	BDL	93.8	BDL
		334	118.6	19.8	BDL	6.6	14.0	BDL	66.6	BDL
		335	79.0	11.2	11.5	BDL	17.8	BDL	84.7	BDL
6	f	406	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
		407	29.5	16.9	BDL	BDL	14.0	BDL	7.4	BDL
		408	79.5	BDL	BDL	BDL	14.0	BDL	164.7	BDL
		409	144.4	14.1	10.4	BDL	14.0	BDL	27.2	BDL
		410	75.7	BDL	6.0	BDL	14.0	BDL	26.5	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 24.41 pg/mL for Leptin, and 4.88 pg/mL for all other analytes).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G9

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	RANTES Duodenum pg/mL	TNF-α Duodenum pg/mL	IL-18 Duodenum pg/mL	Eotaxin Duodenum pg/mL	Leptin Duodenum pg/mL	VEGF Duodenum pg/mL	GRO/KC Duodenum pg/mL
1	f	31	124.2	BDL	111.8	BDL	81.60	67.6	BDL
		32	72.6	BDL	507.4	26.7	379.00	41.4	BDL
		33	35.0	BDL	31.0	BDL	BDL	25.3	BDL
		34	20000.0	10.1	2717.7	99.4	396.40	175.8	28.0
		35	2004.8	BDL	411.1	13.7	BDL	39.6	BDL
2	f	106	97.0	BDL	64.4	BDL	BDL	25.3	BDL
		107	14.2	12.7	64.4	35.8	372.80	81.7	BDL
		108	71.1	5.0	322.5	31.2	169.50	46.0	37.0
		109	20000.0	BDL	1689.2	93.3	304.00	124.7	70.2
		110	366.4	52.9	1355.0	BDL	263.00	19.0	71.8
3	f	181	304.5	BDL	1182.0	47.4	46.50	92.3	BDL
		182	283.6	BDL	354.5	4.9	BDL	61.1	BDL
		183	3407.3	BDL	549.3	BDL	66.40	52.7	11.9
		184	1494.5	BDL	153.9	BDL	82.60	26.0	BDL
		185	3810.1	BDL	1212.3	39.7	34.30	152.6	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 24.41 pg/mL for Leptin, and 4.88 pg/mL for all other analytes).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G9

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex	Animal	RANTES Duodenum pg/mL	TNF-α Duodenum pg/mL	IL-18 Duodenum pg/mL	Eotaxin Duodenum pg/mL	Leptin Duodenum pg/mL	VEGF Duodenum pg/mL	GRO/KC Duodenum pg/mL
4	f	256	823.1	6.8	411.9	17.8	48.10	30.3	18.2
		257	20000.0	BDL	642.8	63.3	74.80	103.4	65.2
		258	1291.0	6.8	269.8	10.7	38.50	BDL	BDL
		259	20000.0	6.5	1292.3	57.2	92.50	75.9	47.9
		260	20000.0	10.0	993.0	55.5	43.80	64.1	BDL
5	f	331	20000.0	20.8	2235.6	79.4	409.80	38.9	161.6
		332	523.1	BDL	134.1	BDL	10.60	BDL	BDL
		333	1244.6	8.5	1089.9	68.2	142.70	118.5	15.6
		334	20000.0	8.6	1417.5	71.5	74.30	88.7	54.9
		335	1024.2	10.4	859.1	28.6	46.50	8.9	BDL
6	f	406	19.8	BDL	246.0	BDL	BDL	BDL	BDL
		407	20000.0	BDL	2114.0	55.9	141.20	41.4	50.2
		408	9002.4	BDL	738.3	BDL	BDL	57.2	BDL
		409	20000.0	BDL	2444.1	74.6	575.50	27.5	39.1
		410	6526.7	BDL	618.9	4.9	BDL	47.2	BDL

BDL = Below detectable level (i.e., less than the lowest standard in the Milliplex kit, which was 24.41 pg/mL for Leptin, and 4.88 pg/mL for all other analytes).

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G10

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex		IL-1α Duodenum pg/mL	IL-1ß Duodenum pg/mL	IL-2 Duodenum pg/mL	IL-4 Duodenum pg/mL	IL-6 Duodenum pg/mL	MCP-1 Duodenum pg/mL	RANTES Duodenum pg/mL	IL-18 Duodenum pg/mL
1	f	Mean	230.55	53.33	20.27	11.33	19.33	17.10	4447.32	755.80
		S.D.	99.61	36.25	14.22	2.72	12.27	11.38	8734.24	1114.63
		N	4	4	3	3	4	4	5	5
2	f	Mean	284.58	88.00	16.64	17.13	17.33	25.80	4109.74	699.10
		S.D.	110.52	80.77	6.24	9.82	4.49	16.55	8883.96	767.79
		N	5	4	5	3	4	3	5	5
3	f	Mean	251.80	97.20	35.74	12.54	13.76	17.17	1860.00	690.40
		S.D.	119.14	67.54	7.72	3.78	8.40	9.21	1675.95	483.38
		N	5	5	5	5	5	3	5	5
4	f	Mean	467.70	119.38	27.46	14.44	22.42	57.44	12422.82	721.96
		S.D.	178.78	38.49	13.95	6.36	8.75	13.87	10376.80	420.00
		N	5	5	5	5	5	5	5	5
5	f	Mean	603.58*	87.44	24.90	9.30	16.95	79.93	8558.38	1147.24
		S.D.	233.34	48.61	13.89	2.91	9.43	11.85	10447.99	769.79
		N	5	5	5	3	4	4	5	5
6	f	Mean	652.50*	82.28	15.50	8.20	14.00	56.45	11109.78	1232.26
		S.D.	181.04	47.24	.	.	0.00	72.75	8753.65	979.66
		N	4	4	2	2	4	4	5	5

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G10

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex		Eotaxin Duodenum pg/mL	Leptin Duodenum pg/mL	VEGF Duodenum pg/mL	G-CSF Duodenum pg/mL	GM-CSF Duodenum pg/mL	IFN-γ Duodenum pg/mL	IL-10 Duodenum pg/mL	IL-12p70 Duodenum pg/mL
1	f	Mean	46.60	285.667	69.94	.	.	46.45	158.30	6.70
		S.D.	46.19	176.941	61.11	.	.	.	.	.
		N	3	3	5	0	0	2	1	1
2	f	Mean	53.43	277.325	59.34	.	9.25	6.80	44.00	13.57
		S.D.	34.60	84.968	43.98	.	.	.	.	9.34
		N	3	4	5	0	2	1	2	3
3	f	Mean	30.67	57.450	76.94	.	7.80	14.88	177.97	11.75
		S.D.	22.64	21.358	48.46	.	.	8.62	12.15	.
		N	3	4	5	0	1	4	3	2
4	f	Mean	40.90	59.540	68.43	.	8.10	9.50	36.26	14.15
		S.D.	24.63	23.133	30.28	.	.	3.98	9.72	6.15
		N	5	5	4	0	2	3	5	4
5	f	Mean	61.93	136.780	63.75	.	.	9.50	127.10	9.60
		S.D.	22.71	160.118	49.15	.	.	3.98	.	.
		N	4	5	4	0	0	3	1	2
6	f	Mean	45.13	358.350	43.33	.	.	14.80	.	8.30
		S.D.	36.08	.	12.41	.	.	.	.	.
		N	3	2	4	0	0	1	0	1

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G10

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary Cytokine/Chemokine Analysis of Duodenum Homogenates

Day: 91 relative to Start Date

Group	Sex		IL-13 Duodenum pg/mL	IL-17 Duodenum pg/mL	IL-5 Duodenum pg/mL	IP-10 Duodenum pg/mL	MIP-1α Duodenum pg/mL	TNF-α Duodenum pg/mL	GRO/KC Duodenum pg/mL
1	f	Mean	6.40	.	9.80	6.70	5.10	10.10	28.00
		S.D.	.	.	.	.	.	.	.
		N	1	0	1	1	1	1	1
2	f	Mean	6.40	.	4.90	.	5.00	23.53	59.67
		S.D.	.	.	.	.	.	25.72	19.65
		N	1	0	1	0	1	3	3
3	f	Mean	8.00	66.30	24.75	6.70	5.53	.	11.90
		S.D.	1.99	.	.	.	0.50	.	.
		N	4	1	2	1	3	0	1
4	f	Mean	13.40	.	7.65	.	6.35	7.53	43.77
		S.D.	6.95	.	.	.	.	1.66	23.77
		N	3	0	2	0	2	4	3
5	f	Mean	6.40	.	6.60	14.40	7.00	12.08	77.37
		S.D.	.	.	.	.	.	5.88	75.55
		N	1	0	1	1	1	4	3
6	f	Mean	.	.	.	.	.	.	44.65
		S.D.	.	.	.	.	.	.	.
		N	0	0	0	0	0	0	2

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table G11

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water Fischer Rats

Protein Concentration of Tissue Homogenates Used for Assay
of 8-Isoprostane and Cytokine Levels:

Protein (µg/mL Homogenate)

Animal ID	Duodenum	Oral Cavity
1F 31	5059	3853
1F 32	a	3818
1F 33	5234	3715
1F 34	4664	3804
1F 35	4459	3820
Mean	**4854**	**3802**
S.D.	**355**	**52**

Animal ID	Duodenum	Oral Cavity
2F 106	5234	3824
2F 107	6472	3970
2F 108	5022	3918
2F 109	4483	3798
2F 110	5155	3993
Mean	**5273**	**3901**
S.D.	**731**	**87**

Animal ID	Duodenum	Oral Cavity
3F 181	5963	3720
3F 182	5479	3626
3F 183	5126	3700
3F 184	a	3773
3F 185	5365	3693
Mean	**5483**	**3702**
S.D.	**352**	**53**

Animal ID	Duodenum	Oral Cavity
4F 256	4499	3917
4F 257	4611	3578
4F 258	5126	3639
4F 259	a	3906
4F 260	5505	3637
Mean	**4935**	**3735**
S.D.	**468**	**163**

Animal ID	Duodenum	Oral Cavity
5F 331	5588	3553
5F 332	4763	3704
5F 333	5112	a
5F 334	5387	3982
5F 335	6247	3778
Mean	**5419**	**3754**
S.D.	**557**	**178**

Animal ID	Duodenum	Oral Cavity
6F 406	4823	3801
6F 407	6771	3975
6F 408	4807	3761
6F 409	4856	3678
6F 410	4815	3800
Mean	**5214**	**3803**
S.D.	**870**	**109**

a = Insufficient sample
Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Attachment G1

Manufacturer’s Instructions for the Ferritin, Transferrin, 8-Isoprostane, and 8-OHdG ELISAs

Ferritin (Rat) ELISA

For the quantitative determination of ferritin in serum or plasma of rats

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	41-FERRT-E01
Size:	96 wells
Version:	2 1.2 - November 22, 2010

ALPCO Diagnostics

26G Keewaydin Drive • Salem, NH 03079
Phone: (800) 592-5726 • Fax: (603) 898-6854
www.alpco.com • Email: web@alpco.com

INTENDED USE

The Ferritin (Rat) ELISA kits are highly sensitive two-site enzyme linked immunoassays (ELISA) for measuring ferritin in serum and plasma of rats.

INTRODUCTION

Ferritin is a water-soluble, iron storage protein. Serum ferritin levels are said to be useful in the study of iron deficiency anemia, metabolism disorders, and malignant tumors. Ferritin may also be an acute-phase protein and is often elevated in the course of disease.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the ferritin present in samples reacts with the anti-ferritin antibodies which have been adsorbed to the surface of polystyrene microplate wells. After the removal of unbound proteins by washing, anti-ferritin antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme labeled antibodies form complexes with the previously bound ferritin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme correlates directly with the concentration of ferritin in the sample tested; the absorbance at 450 nm is a measure of the concentration of ferritin in the sample. The quantity of ferritin in the sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

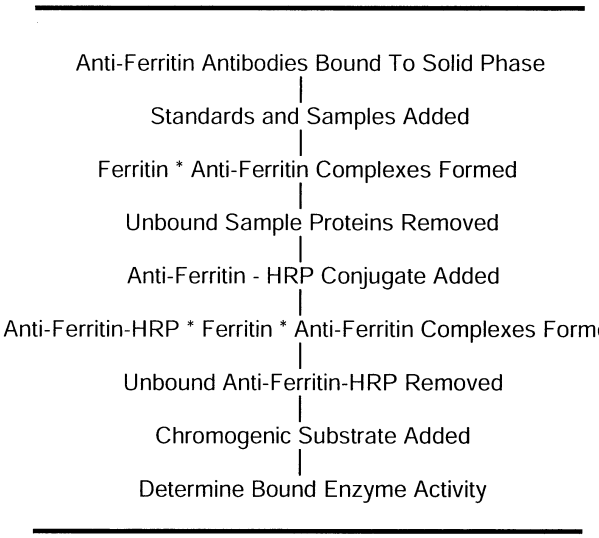


Figure 1.

REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE (assay buffer)
One bottle containing 50 ml of a 5X concentrated Diluent (assay buffer).

2. WASH SOLUTION CONCENTRATE
One bottle containing 50 ml of a 20X concentrated Wash solution.

3. ENZYME ANTIBODY CONJUGATE 100X
One vial containing 150 µl of affinity purified anti-rat ferritin antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN SUBSTRATE SOLUTION
One vial containing 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION
One vial containing 12 ml of 0.3 M sulfuric acid.
WARNING: Avoid contact with skin.

6. ANTI-RAT FERRITIN MICROPLATE
Twelve removable eight (8) well microplate strips in well holder frame. Each well is coated with affinity purified anti-rat ferritin.

7. RAT FERRITIN CALIBRATOR
One vial containing a rat ferritin Calibrator.

8. REFERENCE SERUM
One vial containing a rat Reference Serum. (See Product Profile sheet enclosed with kit.)

REAGENT PREPARATION

1. DILUENT CONCENTRATE
The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with deionized water (1 part Diluent concentrate, 4 parts deionized water).

2. WASH SOLUTION CONCENTRATE
The Wash solution supplied is a 20X concentrate and must be diluted 1:20 with deionized water (1 part Wash concentrate, 19 parts deionized water). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. ENZYME ANTIBODY CONJUGATE
Prepare the required amount of working Conjugate solution for each microplate strip by adding 10 µl of Enzyme Antibody Conjugate to 990 µl of 1X Diluent for each strip to be used. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN SUBSTRATE SOLUTION
Ready to use as supplied.

5. STOP SOLUTION
Ready to use as supplied.

6. ANTI-RAT FERRITIN MICROPLATE
Ready to use as supplied. Unseal Microplate pouch and remove plate from pouch. Remove all strips and wells that **will not** be used from the well holder frame, place back in pouch along with desiccant pack, and reseal.

7. RAT FERRITIN CALIBRATOR

The rat ferritin Calibrator should be frozen in aliquots. It is at a concentration of 2.7 µg/ml. **Rat ferritin Standards need to be prepared immediately prior to use (see chart below).** Mix well between each step. Avoid foaming.

Standards	ng/ml	Volume Added to 1X Diluent	Volume of 1X Diluent
6	400	100 µl of rat ferritin Calibrator	575 µl
5	200	300 µl of Standard 6	300 µl
4	100	300 µl of Standard 5	300 µl
3	50	300 µl of Standard 4	300 µl
2	25	300 µl of Standard 3	300 µl
1	12.5	300 µl of Standard 2	300 µl
0	0		500 µl

8. REFERENCE SERUM

The Reference Serum should be diluted as appropriate to fit within the standard curve range.

STORAGE AND STABILITY

The expiry date for the package is stated on the box label.

1. DILUENT

The 5X Diluent concentrate is stable until the expiry date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. WASH SOLUTION

The 20X Wash solution concentrate is stable until the expiry date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. ENZYME ANTIBODY CONJUGATE

Undiluted horseradish peroxidase anti-ferritin conjugate should be stored at 4-8°C and **diluted immediately prior to use**. The working conjugate solution is stable for up to 8 hours.

4. CHROMOGEN SUBSTRATE SOLUTION

The Chromogen Substrate solution should be stored at 4-8°C and is stable until the expiry date.

5. STOP SOLUTION

The Stop solution should be stored at 4-8°C and is stable until the expiry date.

6. ANTI-RAT FERRITIN MICROPLATE

Anti-rat ferritin coated wells are stable until the expiry date and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. RAT FERRITIN CALIBRATOR

Long Term Storage: Upon receipt, store the Calibrator in frozen aliquots. They will be stable until the expiry date. Short Term Storage: The Calibrator is stable for up to 14 days at 4°C. The working Standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

8. REFERENCE SERUM

The Reference Serum is stable until expiry.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the Standard solutions should be within 20% of the expected values.

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis; excessive hemolysis can impact the results. Assay immediately or store samples in aliquots at -20°C. Avoid repeated freeze-thaw cycles.

1. Precautions For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives No additives or preservatives are necessary to maintain the integrity of the sample. Avoid azide contamination.

3. Known Interfering Substances Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.

MATERIALS PROVIDED - See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (10 µl -1,000 µl) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Deionized or distilled water
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer
- Centrifuge
- Anticoagulant – for collection of plasma samples

ASSAY PROTOCOL

DILUTION OF REFERENCE SERUM AND SAMPLES

The assay for quantification of ferritin in plasma/serum requires that the test samples and Reference serum be diluted before use. For a single step determination a dilution of 1:40 is appropriate for most plasma/serum samples. A lesser or greater dilution might be required for absolute quantification of samples yielding results outside the range of the standard curve. **If unsure of sample level, it is highly recommended to perform a serial dilution with one or two representative samples before running the entire plate.**

1. To prepare a 1:40 dilution of sample, transfer 10 µl of sample to 390 µl of 1X Diluent. This yields a 1:40 dilution. Mix thoroughly.

PROCEDURE

1. Bring all reagents to room temperature before use.

2. Pipette 100 µl of

Standard 0 (0 ng/ml) in duplicate
Standard 1 (12.5 ng/ml) in duplicate
Standard 2 (25 ng/ml) in duplicate
Standard 3 (50 ng/ml) in duplicate
Standard 4 (100 ng/ml) in duplicate
Standard 5 (200 ng/ml) in duplicate
Standard 6 (400 ng/ml) in duplicate

3. Pipette 100 µl of the prediluted samples and eference serum (in duplicate) into the predesignated wells.

4. Incubate the microplate at room temperature for sixty (60 +/- 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash solution and aspirate. Repeat three times, for a total of four washes. If washing manually - completely fill wells with 1X Wash solution, invert the plate, and then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual solution. Repeat three times for a total of four washes.

7. Pipette 100 µl of appropriately diluted Enzyme Antibody Conjugate to each well. Incubate at room temperature for ten (10 +/- 2) minutes. Keep plate covered, level, and in the dark during the incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µl of Chromogen Substrate solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100 µl of Stop solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to two hours after the addition of the Stop solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Substract the average background value from the test values for each sample.

2. Using the results observed for the Standards construct a standard curve. The appropriate curve fit is that of a four parameter logistics curve. A second order polynomial (quadratic) or other curve fit may also be used.

3. Interpolate test sample values from the standard curve. Correct for sera dilution factor to arrive at the ferritin concentration in the original sample.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function; cleanliness of glassware; quality of deionized water; and accuracy of reagent and sample pipettings, washing technique, and incubation times/temperatures.

3. Do not mix or substitute reagents with those from other lots or sources.

Transferrin (Rat) ELISA

For the quantitative determination of transferrin in rat serum and plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	41-TRART-E01
Size:	96 wells
Version:	2 L11.0 - ALPCO October 26, 2010

ALPCO Diagnostics

26G Keewaydin Drive • Salem, NH 03079
Phone: (800) 592-5726 • Fax: (603) 898-6854
www.alpco.com • Email: web@alpco.com

INTENDED USE

The Transferrin (Rat) ELISA test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring transferrin in serum and plasma of rats.

INTRODUCTION

Transferrin is a metal-combining protein that binds reversibly to acid-soluble iron in plasma. Its function is to transport iron to the bone marrow, and to tissue storage organs such as the liver. Transferrin also participates in the regulation and control of iron absorption and protects against iron intoxication. Like haptoglobin, the carrier of hemoglobin, transferrin is synthesized in the liver, but unlike haptoglobin, transferrin is returned to the circulation after unloading its iron in the reticuloendothelial system. This ELISA kit can be used to measure transferrin in serum and plasma.

PRINCIPLE OF THE ASSAY

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the transferrin present in the sample reacts with the anti-transferrin antibodies which have been adsorbed to the surface of polystyrene microplate wells. After the removal of unbound sample proteins by washing, anti-transferrin antibodies conjugated with horseradish peroxidase (HRP) are added. These enzyme-labeled antibodies form complexes with the previously bound sample transferrin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme correlates directly with the concentration of transferrin in the sample tested; the absorbance at 450 nm is a measure of the concentration of transferrin in the sample. The quantity of transferrin in the sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

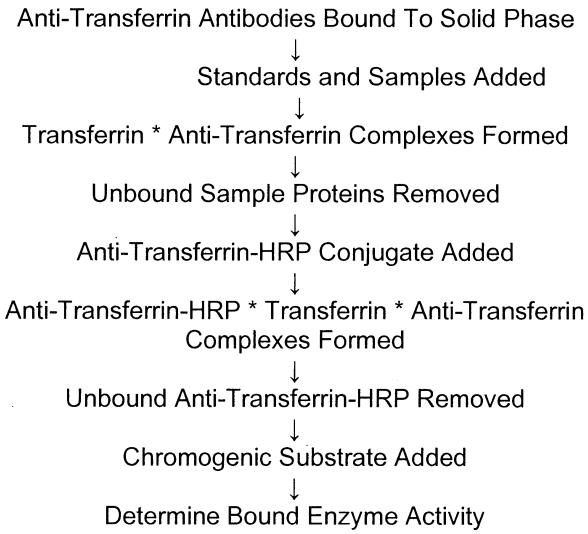


Figure 1.

REAGENTS (Quantities sufficient for 96 determinations)

1. DILUENT CONCENTRATE (assay buffer)
One bottle containing 50 ml of a 5X concentrated Diluent (assay buffer).

2. WASH SOLUTION CONCENTRATE
One bottle containing 50 ml of a 20X concentrated Wash solution.

3. ENZYME ANTIBODY CONJUGATE 100X
One vial containing 150 µl of affinity purified anti-rat transferrin antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN SUBSTRATE SOLUTION
One vial containing 12 ml of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION
One vial containing 12 ml of 0.3 M sulfuric acid. **WARNING: Avoid contact with skin.**

6. ANTI-RAT TRANSFERRIN ELISA MICROPLATE
Twelve removable eight (8) well microplate strips in well holder frame. Each well is coated with affinity purified anti-rat transferrin.

7. RAT TRANSFERRIN CALIBRATOR
One vial containing a lyophilized rat transferrin Calibrator.

FOR RESEARCH USE ONLY

REAGENT PREPARATION

1. DILUENT CONCENTRATE
The Diluent supplied is a 5X concentrate and must be diluted 1:5 with distilled or deionized water (1 part buffer concentrate, 4 parts deionized water).

2. WASH SOLUTION CONCENTRATE
The Wash solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or deionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals (1 part buffer concentrate, 19 parts deionized water).

3. ENZYME ANTIBODY CONJUGATE
The required amount of working conjugate solution for each microplate is prepared by adding 10 µl Enzyme Antibody Conjugate to 990 µl of 1X Diluent for each test strip to be used. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN SUBSTRATE SOLUTION
Ready to use as supplied.

5. STOP SOLUTION
Ready to use as supplied.

6. ANTI-RAT TRANSFERRIN ELISA MICROPLATE
Ready to use as supplied. Unseal microplate pouch and remove plate. Remove all strips and wells

that WILL NOT be used from the well holder frame, place back in pouch along with desiccant pack, and reseal.

7. RAT TRANSFERRIN CALIBRATOR
Add 1.0 ml of distilled or deionized water to the rat transferrin Calibrator and mix gently until dissolved. The Calibrator is now at a concentration of 10.56 µg/ml **(the reconstituted Calibrator should be frozen in aliquots if future use is intended). Rat transferrin Standards need to be prepared immediately prior to use (see chart below).** Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to 1X Diluent	Volume of 1X Diluent
7	400	36 µl Rat Transferrin Calibrator	914 µl
6	200	300 µl Standard 7	300 µl
5	100	300 µl Standard 6	300 µl
4	50	300 µl Standard 5	300 µl
3	25	300 µl Standard 4	300 µl
2	12.5	300 µl Standard 3	300 µl
1	6.25	300 µl Standard 2	300 µl
0	0		500 µl

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.

1. DILUENT
The 5X Diluent concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8°C.

2. WASH SOLUTION
The 20X Wash solution concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4-8°C.

3. ENZYME ANTIBODY CONJUGATE
Undiluted horseradish peroxidase anti-transferrin Conjugate should be stored at 4-8°C and **diluted immediately prior to use.** The working Conjugate solution is stable for up to 8 hours.

4. CHROMOGEN SUBSTRATE SOLUTION
The Chromogen Substrate solution should be stored at 4-8°C and is stable until the expiration date.

5. STOP SOLUTION
The Stop solution should be stored at 4-8°C and is stable until the expiration date.

6. ANTI-RAT TRANSFERRIN ELISA MICROPLATE
Anti-rat transferrin coated wells are stable until the expiration date, and should be stored at 4-8°C in the sealed foil pouch with desiccant pack.

7. RAT TRANSFERRIN CALIBRATOR
The lyophilized rat transferrin Calibrator should be stored at 4°C or frozen until reconstituted. The reconstituted Calibrator should be stored frozen in aliquots (multiple freeze/thaw cycles should be avoided). The working Standard solutions should be prepared

immediately prior to use and are stable for up to 8 hours.

INDICATIONS OF INSTABILITY
If the test is performing correctly, the results observed with the Standard solutions should be within 20% of the expected values.

SAMPLE COLLECTION AND HANDLING

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis; excessive hemolysis can impact test results. Assay immediately or store samples in aliquots at -20°C. Avoid repeated freeze/thaw cycles.

1. Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

3. Known interfering substances

Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.

MATERIALS PROVIDED - See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (5 µl to 1 ml) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Distilled or deionized water
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Anticoagulant (for collection of plasma)
- Vortex mixer
- Centrifuge

ASSAY PROTOCOL

DILUTION OF SERUM SAMPLES

The assay for quantification of transferrin requires that each sample be diluted before use. A 1:40,000 dilution is appropriate for most serum or plasma samples. A lesser or greater dilution might be required for absolute quantification of samples that yield results outside the range of the standard curve. **If unsure of sample concentration, it is highly recommended to test a serial dilution with one or two representative samples before running the entire plate.**

1. To prepare a 1:40,000 dilution of sample, transfer 5 µl of sample to 995 µl of 1X Diluent. This gives yields a 1:200 dilution. Next, dilute the 1:200 samples by transferring 5 µl to 995 µl of 1X Diluent. This yields a 1:40,000 dilution of the sample. Mix thoroughly at each stage.

PROCEDURE

1. **Bring all reagents to room temperature before use.**

2. Pipette 100 µl of
   Standard 0 (0 ng/ml) in duplicate
   Standard 1 (6.25 ng/ml) in duplicate
   Standard 2 (12.5 ng/ml) in duplicate
   Standard 3 (25 ng/ml) in duplicate
   Standard 4 (50 ng/ml) in duplicate
   Standard 5 (100 ng/ml) in duplicate
   Standard 6 (200 ng/ml) in duplicate
   Standard 7 (400 ng/ml) in duplicate
3. Pipette 100 µl of the diluted samples (in duplicate) into the pre-designated wells.
4. Incubate the microplate at room temperature (16-25°C) for fifteen (15 ± 2) minutes. Keep plate covered and level during incubation.
5. Following incubation, aspirate the contents of the wells.
6. Completely fill each well with appropriately diluted Wash solution and aspirate. Repeat three times, for a total of four washes. If washing manually, completely fill wells with Wash solution, invert the plate, and pour/shake out the contents into a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual solution. Repeat three times for a total of four washes.
7. Pipette 100 µl of appropriately diluted Enzyme Antibody Conjugate to each well. Incubate at 22°C (room temperature) for fifteen (15 ± 2) minutes. Keep plate covered, in the dark, and level during incubation.
8. Wash and blot the wells as described in Steps 5 and 6.
9. Pipette 100 µl of Chromogen Substrate solution into each well.
10. Incubate in the dark at room temperature for precisely ten (10) minutes.
11. After ten minutes, add 100 µl of Stop solution to each well.
12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to air.

STABILITY OF THE FINAL REACTION MIXTURE

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background (0 ng/ml Standard) value from the test values for each sample.

2. Using the results observed for the standards construct a standard curve. The appropriate

curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fit may also be used.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the transferrin concentration in the original sample.

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, thoroughness of washing, and accuracy of reagent and sample pipettings and incubation time/temperature.

3. Do not mix or substitute reagents with those from other lots or sources.

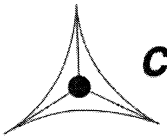
Product Manual

OxiSelect™ 8-iso-Prostaglandin F2α ELISA Kit

Catalog Numbers

STA-337 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

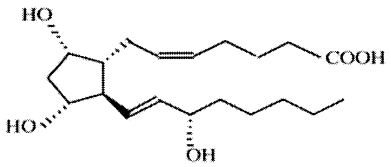


CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Lipid peroxidation is a well-defined mechanism of cellular damage in animals and plants. Lipid peroxides are unstable indicators of oxidative stress in cells that decompose to form more complex and reactive compounds such as isoprostanes. The isoprostanes are a type of eicosanoids produced non-enzymatically through the oxygen radical induced peroxidation of tissue phospholipids and lipoproteins. Isoprostanes are prostaglandin-like compounds that appear in normal plasma and urine samples, but are elevated by oxidative stress in tissue, plasma, and urine.

8-iso-Prostaglandin F2α (also known as 8-epi-PGF2α, 8-isoprostane, or 15-isoprostane F2t), is an isoprostane that has been shown to be useful for the assessment of oxidative stress *in vivo*. It is produced in membrane phospholipids from non-cyclooxygenase and cyclooxygenase peroxidation pathways derived from arachidonic acid. 8-iso-Prostaglandin F2α (8-iso-PGF2α) is a potent vasoconstrictor, a mutagen in 3T3 cells as well as vascular smooth muscle cells, and also a possible pathophysiological mediator that can alter membrane integrity. It has been implicated in atherogenesis and elevated levels are associated with hepatorenal syndrome, rheumatoid arthritis, carcinogenesis, as well as atherosclerosis. 8-iso-PGF2α circulates in the plasma and is excreted in the urine. 8-iso PGF2α circulates as an esterified LDL Phospholipid and as a free acid. The total normal plasma 8-iso PGF2α is about 40-100 pg/mL and about 190 pg/mg of creatine. Methods for determining total 8-iso PGF2α usually require alkaline hydrolysis of 8-iso PGF2α esters from tissues followed by extractions, phase separations and thin layer chromatography.



8-iso-Prostaglandin F2α (8-iso-PGF2α)

Cell Biolabs' OxiSelect™ 8-iso-Prostaglandin F2α ELISA Kit is an enzyme immunoassay developed for rapid detection and quantification of 8-iso-Prostaglandin F2α. The quantity of 8-iso-PGF2α in samples is determined by comparing its absorbance with that of a known 8-iso-PGF2α standard curve. Each kit provides sufficient reagents to perform up to 96 assays, including the standard curve and unknown phospholipids samples.

Assay Principle

Cell Biolabs' 8-iso-PGF2α kit is a competitive enzyme-linked immunoassay (ELISA) for determining levels of 8-iso-PGF2α in a variety of biological samples such as plasma, urine, serum, or tissue extracts. An antibody to 8-iso-PGF2α is incubated in pre-coated microtiter plate wells. Upon washing, 8-iso-PGF2α standards or treated samples are mixed with an 8-iso-PGF2α-HRP conjugate and added simultaneously to the wells. The unconjugated, or free 8-iso-PGF2α and 8-iso-PGF2α-HRP conjugate compete for binding to the antibody bound to the plate. After this brief incubation and wash, a substrate to the HRP is added. The HRP activity results in color development that is directly proportional to the amount of 8-iso-PGF2α conjugate bound to the plate and inversely proportional to the amount of free 8-iso-PGF2α in the samples or standards. The 8-iso-PGF2α content in an unknown

sample is determined by comparing with the known predetermined standard curve. Please read the complete kit insert prior to performing the assay.

Related Products

1. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
2. STA-334: OxiSelect™ HNE-His Adduct ELISA Kit
3. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
4. STA-341- OxiSelect™ Catalase Activity Assay Kit
5. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
6. STA-320: OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)
7. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG Quantitation)
8. STA-350: OxiSelect™ Comet Assay (3-Well Slides)
9. STA-345: OxiSelect™ ORAC Activity Assay
10. STA-346: OxiSelect™ HORAC Activity Assay

Kit Components

1. Goat Anti-Rabbit Antibody Coated Plate (Part No. 250001): One 96-well strip plate.
2. Anti-8-iso-PGF2α Antibody (Part No. 233701): One 20 µL tube of anti-8-iso-PGF2α rabbit IgG.
3. Sample Diluent (Part No. 233702): One 50 mL bottle.
4. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
5. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
6. Stop Solution (Part. No. 310808): One 12 mL bottle.
7. 8-iso-PGF2α Standard (Part No. 233703): One 25 µL tube of 200 µg/mL 8-iso-PGF2α in DMSO.
8. 8-iso-PGF2α-HRP Conjugate (Part No. 233704): One 70 µL tube of 8-iso-PGF2α-HRP conjugate.

Materials Not Supplied

1. Protein samples such as purified protein, plasma, serum, cell lysate
2. Deionized water
3. 5 µL to 1000 µL adjustable single channel precision micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Bottles, flasks, and conical or microtubes necessary for reagent preparation
6. Reagents and materials necessary for sample extraction and purification
7. Multichannel micropipette reservoir
8. Plate orbital shaker or rotator
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, store the Anti-8-iso-PGF2α Antibody, 8-iso-PGF2α-HRP Conjugate, and 8-iso-PGF2α Standard at -20°C. Make aliquots as necessary to avoid freeze/thaw cycles. Store all other kit components at 4°C until their expiration dates. Any partial or unused components should return to their proper storage temperatures.

Safety Considerations

1. Some kit components contain azide, which can react with copper or lead piping. Flush with large volumes of water when disposing of reagents.
2. Some kit reagents are caustic or hazardous and should be handled accordingly.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-8-iso-PGF2α Antibody: Immediately before use, dilute the Anti-8-iso-PGF2α Antibody 1:1000 with Sample Diluent.
- 8-iso-PGF2α-HRP Conjugate: Immediately before use, dilute the conjugate 1:80 with Sample Diluent. Only prepare enough of the diluted conjugate for the number of wells immediately used.
- Substrate Solution: Prior to use, warm the Substrate Solution to room temperature.

Note: Do not store diluted Anti-8-iso-PGF2α Antibody, 8-iso-PGF2α-HRP Conjugate, or 8-iso-PGF2α Standard solutions.

Preparation of Samples

Hydrolysis of lipoprotein or phospholipid coupled 8-iso-Prostaglandin F2α (8-iso-PGF2α) is required to measure both free and esterified isoprostane. To hydrolyze this ester bond, the sample is usually treated with 2N NaOH at 45 °C for 2 hours.

Serum, plasma, tissue lysate samples:

Use 1 part of 10N NaOH for every 4 parts of liquid sample. After incubation at 45 °C for 2 hours, add 100 µL of concentrated (12.1N) HCl per 500 µL of hydrolyzed sample. The sample could turn milky after this addition. Centrifuge the samples for 5 minutes at 12,000 rpm in a microcentrifuge. The clear supernatant can be used in the assay or stored at ≤ -20 °C for future use. If necessary check the pH of the neutralized samples. The pH should be in the range of 6-8. If it is not, adjust the pH to this range by adding 1 M Tris stock, pH 7.0, to a final 50 mM Tris.

Urine Sample:

Urine sample is acidified to pH 3.0 by adding 1/10 volume of 1N HCl (Example: Add 100 µL of 1N HCl to 1 mL of urine sample). Acidified urine sample should be further diluted in PBS or Sample Diluent 1:4 to 1:8 before ELISA.

Preparation of 8-iso-PGF2α Standards

1. Prepare fresh standards by diluting the 8-iso-PGF2α Standard from 200µg/mL to 0.2 µg/mL in Sample Diluent for a 1:1000 final dilution. (Example: Add 5 µL of 8-iso-PGF2α Standard stock tube to 4.995 mL of Sample Diluent)
2. Prepare a series of the remaining 8-iso-PGF2α standards according to Table 1.

Standard Tubes	8-iso-PGF2α Standard (µL)	Sample Diluent (µL)	8-iso-PGF2α Standard (pg/mL)
1	5 µL of Standard Stock	4995 µL	200,000
2	250 µL of Tube #1	750 µL	50,000
3	250 µL of Tube #2	750 µL	12,500
4	250 µL of Tube #3	750 µL	3,125
5	250 µL of Tube #4	750 µL	781
6	250 µL of Tube #5	750 µL	195
7	250 µL of Tube #6	750 µL	49
8	0 µL	200 µL	0

Table 1. Preparation of 8-iso-PGF2α Standard Curve.

Note: Do not store diluted 8-iso-PGF2α Standard solutions.

Assay Protocol

Note: Each 8-iso-PGF2α Standard and unknown samples should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

1. Add 100 µL of the diluted Anti-8-iso-PGF2α Antibody to the Goat Anti-Rabbit Antibody Coated Plate. Incubate 1 hour at 25°C on an orbital shaker.
2. Remove the antibody solution from the wells. Wash wells 5 times with 300 µL 1X Wash Buffer per well. After the last wash, empty the wells and tap microwell plate on absorbent pad or paper towel to remove excess wash solution.

   Note: Thorough washing is necessary to remove all of the azide present in the antibody solution.
3. Combine 55 µL of the 8-iso-PGF2α standard or sample and 55 µL of 8-iso-PGF2α-HRP conjugate in a microtube and mix thoroughly. Transfer 100 µL of the combined solution per well. A well containing Sample Diluent can be used as a control. Incubate 1 hour at 25°C on an orbital shaker.
4. Remove the combined solution from the wells. Wash 5 times with 300 µL of 1X Wash Buffer per well. After the last wash, empty wells and tap microwell plate on absorbent pad or paper towel to remove excess wash solution.

5. Add 100 µL of Substrate Solution to each well. Incubate at room temperature for 10-30 minutes on an orbital shaker.
6. Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Results should be read immediately (color will fade over time).
7. Read absorbance of each well on a microplate reader using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical 8-iso-PGF2α results. One should use the data below for reference only. This data should not be used to interpret actual results.

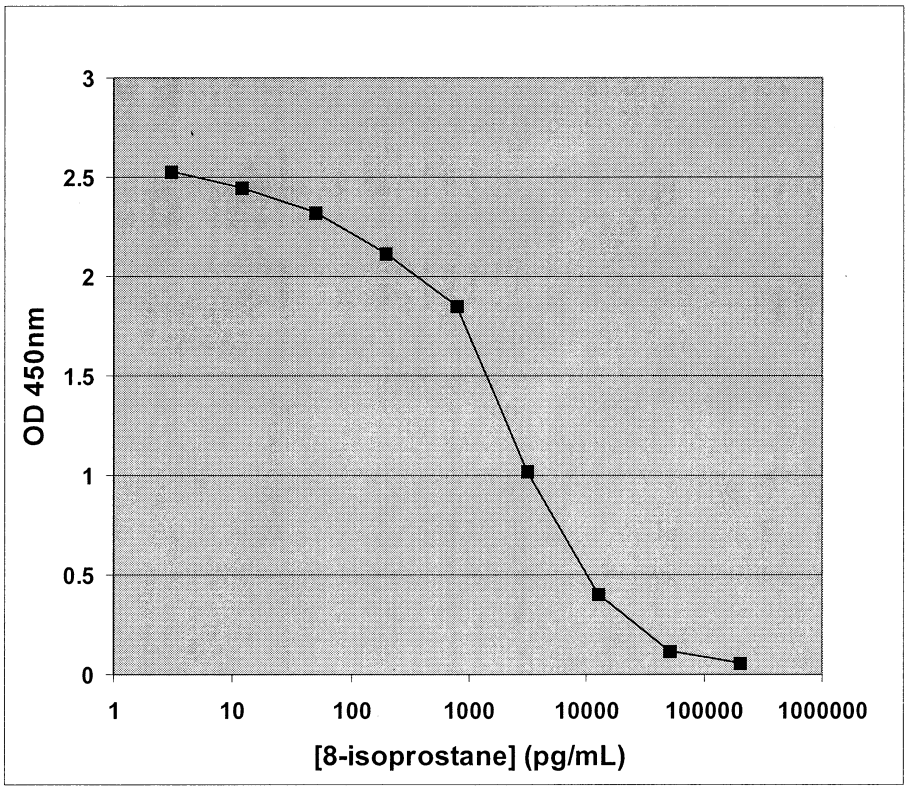


Figure 1: 8-iso-PGF2α ELISA Standard Curve.

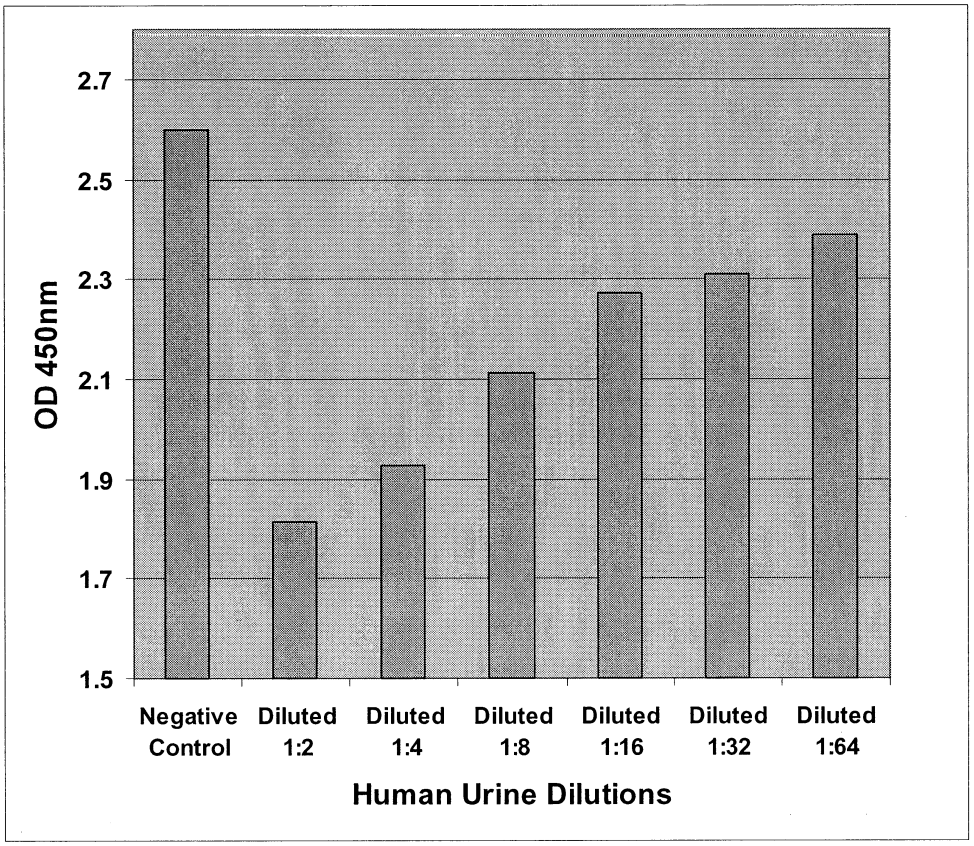


Figure 2: Dilutions of Human Urine tested with 8-iso-PGF2α ELISA.

Cross reactivity of 8-iso-Prostaglandin F2α ELISA Kit

Compounds	Cross Reactivity
8-iso-PGF2α	100%
PGF1α	4.6%
PGF2α	1.85%
PGE1	0.19%
TXB2	0.023%
PGB1	0.02%
PGE3	0.012%
6-keto-PGF1α	0.008%
13,14-dihydro-15-keto-PGF2α	0.008%
6,15-keto-13,14-dihydro-PGF1α	0.005%
8-iso-PGE1	<0.001%
PGA2	<0.001%
PGJ2	<0.001%

References

1. Banerjee, M., Kang, K.H., Morrow, J.D., et al. (1992) *Am. J. Physiol.* 263: H660-H663.
2. Morrow, J.D., Hill, K.E., Burk, R.F., et al. (1990) *Proc. Natl. Acad. Sci. USA.* 87: 9383-9387.
3. Morrow, J.D., Harris, T.M., Roberts, L.J. (1990) *Anal. Biochem.* 184: 1-10.
4. Vacchiano, C.A., and Tempel, G.E. (1994) J. *Appl. Physiol.* 77: 2912-2917.
5. Wang, Z., Ciabattoni, G., Cre'minon, C., et al. (1995) *Pharmacol. Exp. Ther.* 275: 94-100.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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www.cellbiolabs.com

Revised Protocol

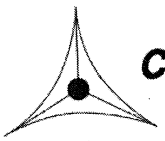
Product Manual

OxiSelect™ Oxidative DNA Damage ELISA Kit (8-OHdG Quantitation)

Catalog Number

STA-320	96 assays
STA-320-5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
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Introduction

Free radicals and other reactive species are constantly generated *in vivo* and cause oxidative damage to biomolecules, a process held in check only by the existence of multiple antioxidant and repair systems as well as the replacement of damaged nucleic acids, proteins and lipids. DNA is probably the most biologically significant target of oxidative attack, and it is widely thought that continuous oxidative damage to DNA is a significant contributor to the age-related development of the major cancers, such as those of the colon, breast, rectum, and prostate. Among numerous types of oxidative DNA damage, the formation of 8-hydroxydeoxyguanosine (8-OHdG) is a ubiquitous marker of oxidative stress. 8-OHdG, one of the oxidative DNA damage byproducts, is physiologically formed and enhanced by chemical carcinogens. During the repair of damaged DNA *in vivo* by exonucleases, the resulting 8-OH-dG is excreted without further metabolism into urine.

Cell Biolabs' Oxidative DNA Damage ELISA Kit is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-OHdG in urine, serum, or other cell or tissue DNA samples. The quantity of 8-OHdG in unknown sample is determined by comparing its absorbance with that of a known 8-OHdG standard curve. The kit has an 8-OHdG detection sensitivity range of 100 pg/mL to 20 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Assay Principle

The Oxidative DNA Damage ELISA kit is a competitive ELISA for the quantitative measurement of 8-OHdG. The unknown 8-OHdG samples or 8-OHdG standards are first added to an 8-OHdG/BSA conjugate preabsorbed EIA plate. After a brief incubation, an anti-8-OHdG monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The 8-OHdG content in unknown samples is determined by comparison with predetermined 8-OHdG standard curve.

Related Products

1. STA-303: OxiSelect™ Nitrotyrosine Immunoblot Kit
2. STA-305: OxiSelect™ Nitrotyrosine ELISA Kit
3. STA-308: OxiSelect™ Protein Carbonyl Immunoblot Kit
4. STA-310: OxiSelect™ Protein Carbonyl ELISA Kit
5. STA-315: OxiSelect™ Protein Carbonyl Spectrophotometric Assay
6. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
7. STA-332: OxiSelect™ MDA ELISA Kit
8. STA-334: OxiSelect™ HNE Adduct ELISA Kit
9. STA-324: OxiSelect™ Oxidative DNA Damage Quantitation Kit (AP Sites)
10. STA-325: OxiSelect™ Oxidative RNA Damage ELISA Kit (8-OHG)

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One strip well 96-well plate.
2. Anti-8-OHdG Antibody (Part No. 232002): One 15 µL vial of anti-8-OHdG.
3. Secondary Antibody, HRP Conjugate (Part No. 10902): One 50 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.
8. 8-OHdG Standard (Part No. 232003): One 100 µL vial of 2 µg/mL 8-OHdG in 1X PBS, 0.1% BSA.

Box 2 (shipped on blue ice packs)

1. 8-OHdG Conjugate (Part No. 232001): One 20 µL vial of 8-OHdG-BSA conjugate at 1.0 mg/mL in PBS.

Materials Not Supplied

1. 8-OHdG samples such as serum, urine, cell or tissue DNA
2. DNA Extraction Kit
3. Sodium Acetate, pH 5.2
4. Tris Buffer, pH7.5
5. Nuclease P1, Alkaline Phosphatase
6. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
7. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
8. Multichannel micropipette reservoir
9. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the **8-OHdG Standard at -20°C** and the **8-OHdG Conjugate at -80°C** to avoid multiple freeze/thaw cycles. Store all other components at 4°C until their expiration dates.

Preparation of Reagents

- 8-OHdG Coated Plate: Dilute the proper amount of 8-OHdG Conjugate (1 mg/mL) to **1 µg/mL** in 1X PBS. Add 100 µL of the **1 µg/mL** 8-OHdG Conjugate to each well and incubate overnight at 4°C. Remove the 8-OHdG coating solution and wash once with dH_2O. Blot plate on paper towels to remove excess fluid. Add 200 µL of Assay Diluent to each well and block for 1 hr at room temperature. Transfer the plate to 4°C and remove the Assay Diluent immediately before use. *Note: 8-OHdG coated plate is not stable. We recommend using it within 24 hrs after coating.*
- 1X Wash Buffer: Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.
- Anti-8-OHdG Antibody and Secondary Antibody: Immediately before use dilute the Anti-8-OHdG Antibody 1:500 and Secondary Antibody 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of 8-OHdG standards in the concentration range of 0 ng/mL – 20 ng/mL by diluting the 8-OHdG Standard in Assay Diluent (Table 1).

Standard Tubes	8-OHdG Standard (µL)	Assay Diluent (µL)	8-OHdG (ng/mL)
1	10	990	20
2	500 of Tube #1	500	10
3	500 of Tube #2	500	5
4	500 of Tube #3	500	2.5
5	500 of Tube #4	500	1.25
6	500 of Tube #5	500	0.625
7	500 of Tube #6	500	0.313
8	500 of Tube #7	500	0.156
9	500 of Tube #8	500	0.078
10	0	500	0

Table 1. Preparation of 8-OHdG Standards

Preparation of Samples

I. Urine or Serum Samples

Clear urine or serum samples can be diluted in Assay Diluent and used directly in the assay. Samples containing precipitates should be centrifuged at 3000 g for 10 minutes, or filtered through 0.45 µm filter, prior to use in the assay.

II. Cell or Tissue DNA Samples:

1. Extract DNA from cell or tissue samples by a desired method or commercial DNA Extraction kit.
2. Dissolve extracted DNA in water at 1-5 mg/mL.
3. Convert DNA sample to single-stranded DNA by incubating the sample at 95°C for 5 minutes and rapidly chilling on ice.
4. Digest DNA sample to nucleosides by incubating the denatured DNA with 5-20 units of nuclease P1 for 2 hrs at 37°C in 20 mM Sodium Acetate, pH 5.2, and following with treatment of 5-10 units of alkaline phosphatase for 1 hr at 37 ^{0}C in 100 mM Tris, pH 7.5.
5. The reaction mixture is centrifuged for 5 minutes at 6000 g and the supernatant is used for the 8-OHdG ELISA assay.

Assay Protocol

1. Prepare and mix all reagents thoroughly before use. Each 8-OHdG sample including unknown and standard should be assayed in duplicate. High content 8-OHdG urine or serum samples should be diluted at least 10-20 fold in Assay Diluent.
2. Add 50 µL of unknown sample or 8-OHdG standard to the wells of the 8-OHdG Conjugate coated plate. Incubate at room temperature for 10 minutes on an orbital shaker.
3. Add 50 µL of the diluted anti-8-OHdG antibody to each well, incubate at room temperature for 1 hour on an orbital shaker.
4. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
5. Add 100 µL of the diluted Secondary Antibody-Enzyme Conjugate to all wells.
6. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash microwell strips 3 times according to step 4 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical Oxidative DNA Damage ELISA results. One should use the data below for reference only. This data should not be used to interpret actual results.

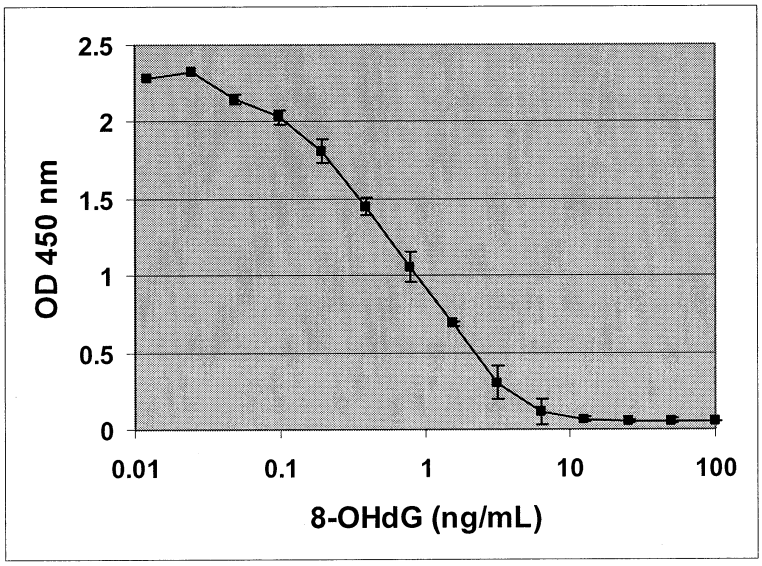

Figure 1: 8-OHdG ELISA Standard Curve.

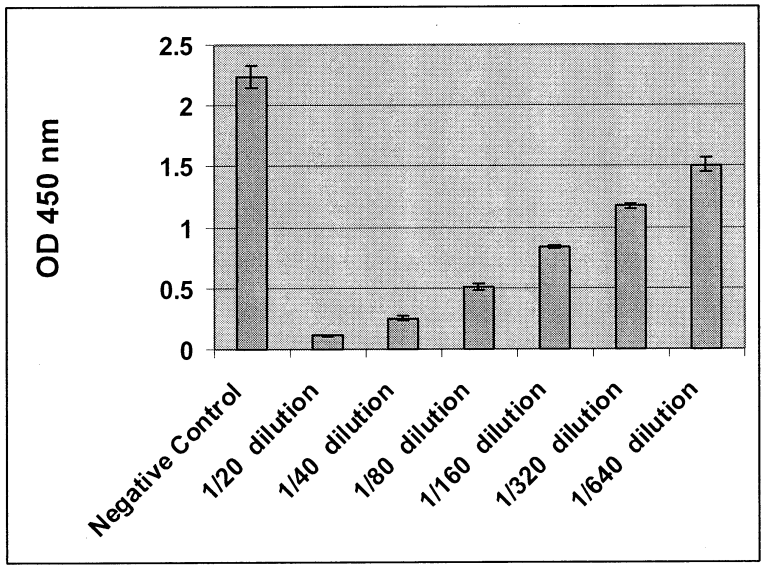

Figure 2: 8-OHdG level in human urine sample.

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2. Shen J, Deininger P, Hunt J. D, and Zhao H. (2007) Cancer **109**, 574-580.
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1. Ksiazek, K. et al. (2008). Impaired response to oxidative stress in senescent cells may lead to acccumulation of DNA damage in mesothelial cells from aged donors. *Biochem. and Biophys. Res. Comm.* **373**:335-339.
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4. Hasegawa, T. et al. (2009*). Suppression of nitrosative and oxidative stress to reduce cardiac allograft vasculopathy. Am.J. Physiol. Hear Circ. Physiol.* 10.1152/ajpheart.00498.2008.
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Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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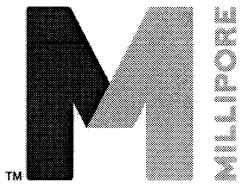
Attachment G2

Manufacturer’s Instructions for the Cytokine/Chemokine Multiplexing



Rat Cytokine / Chemokine

96 Well Plate Assay

Cat. #: RCYTO-80K or
RCYTO-80K-PMX, or
RCYTO-80K-PMX23

MILLIPLEX® MAP

RAT CYTOKINE / CHEMOKINE KIT
96 Well Plate Assay

#RCYTO-80K or
#RCYTO-80K-PMX (14-plex premixed) or
#RCYTO-80K-PMX23 (premixed)

TABLE OF CONTENTS	PAGE

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

"Cytokine" describes a diverse group of soluble proteins and peptides that act as regulators under both normal and pathological conditions to modulate the functional activities of individual cells and tissues. These proteins also mediate interactions between cells directly and regulate processes taking place in the extracellular environment. Cytokines differ from hormones in that they act on a wider spectrum of target cells and are not produced by specialized cells in glands. This includes lymphokines, interferons, colony stimulating factors and chemokines (cytokines with chemotactic activity).

Millipore is proud to announce that the former LINCO*plex* Rat Cytokine/Chemokine Panel now has the MILLIPLEX® MAP optimized format. While you will instantly recognize the quality and reproducibility that you have always trusted, you will also enjoy the enhancements that we have built into MILLIPLEX MAP.

Millipore's MILLIPLEX® MAP Rat Cytokine/Chemokine Panel is to be used for the simultaneous quantification of Eotaxin, G-CSF, GM-CSF, GRO/KC, IFNγ, IP-10, Leptin, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17, IL-18, MCP-1, MIP-1a, RANTES, TNFα and VEGF in rat plasma, serum, and cell/tissue culture supernatant samples. The panel provides quality tools for biomedical researchers who use rat animal models in study of inflammatory diseases.

This kit is for research purposes only.

Please read entire protocol before use.

It is important to use same assay incubation conditions throughout your study.

PRINCIPLE

MILLIPLEX® MAP is based on the Luminex® xMAP® technology — one of the fastest growing and most respected multiplex technologies offering applications throughout the life sciences and capable of performing a variety of bioassays including immunoassays on the surface of fluorescent-coded beads known as microspheres.

- Luminex® uses proprietary techniques to internally color-code microspheres with two fluorescent dyes. Through precise concentrations of these dyes, 100 distinctly colored bead sets can be created, each of which is coated with a specific capture antibody.
- After an analyte from a test sample is captured by the bead, a biotinylated detection antibody is introduced.
- The reaction mixture is then incubated with Streptavidin-PE conjugate, the reporter molecule, to complete the reaction on the surface of each microsphere.
- The microspheres are allowed to pass rapidly through a laser which excites the internal dyes marking the microsphere set. A second laser excites PE, the fluorescent dye on the reporter molecule.
- Finally, high-speed digital-signal processors identify each individual microsphere and quantify the result of its bioassay based on fluorescent reporter signals.

The capability of adding multiple conjugated beads to each sample results in the ability to obtain multiple results from each sample. Open-architecture xMAP® technology enables multiplexing of many types of bioassays reducing time, labor and costs over traditional methods.

STORAGE CONDITIONS UPON RECEIPT

- Recommended storage for kit components is 2 - 8°C.
- Once the standards and controls have been reconstituted, immediately transfer contents into polypropylene vials. **DO NOT STORE RECONSTITUTED STANDARDS OR CONTROLS IN GLASS VIALS**. For long-term storage, freeze reconstituted standards and controls at ≤ -20°C. Avoid multiple (>2) freeze/thaw cycles.
- **DO NOT FREEZE Antibody-Immobilized Beads, Detection Antibodies, and Streptavidin-Phycoerythrin**.

REAGENTS SUPPLIED

Note: Store all reagents at 2 – 8°C

REAGENTS SUPPLIED	CATALOG NUMBER	VOLUME	QUANTITY
Rat Cytokine / Chemokine Standard	LRC-8080	lyophilized	1 vial
Rat Cytokine Quality Controls 1 and 2	LRC-6080	lyophilized	2 vials
Serum Matrix Note: Contains 0.08% Sodium Azide	LMC-SD	lyophilized	1 vial (required for serum and plasma samples only)
Bead Diluent (not provided with premix panel)	LA-BD	4.0 mL	1 vial
Set of one 96-Well Filter Plate with 2 Sealers	MX-PLATE	----------	1 plate 2 sealers
Assay Buffer	L-MAB	30 mL	1 bottle
10X Wash Buffer Note: Contains 0.05% Proclin	L-WB	30 mL	1 bottle
Rat Cytokine Detection Antibodies	LRC-1080-1 or LRC-1080-2 or LRC-1080-3	3.2 mL	1 bottle
Streptavidin-Phycoerythrin	L-SAPE4 *(Use with Cat. # LRC-1080-3)* or L-SAPE6 *(Use with Cat. # LRC-1080-1 & LRC-1080-2)*	3.2 mL	1 bottle
Mixing Bottle (not provided with premixed panel)	----------	----------	1 bottle

Rat Cytokine / Chemokine Antibody-Immobilized Premixed Beads:

Premixed 14-plex Beads	MXRCB-PMX	3.5 mL	1 bottle
Premixed 23-plex Beads	MXRCB-PMX23	3.5 mL	1 bottle

Included Rat Cytokine / Chemokine Antibody-Immobilized Beads are dependent on customizable selection of analytes within the panel (see following table page 4).

Rat Cytokine / Chemokine Antibody-Immobilized Beads:

Bead/Analyte Name	Luminex Bead Region	Customizable 23 Analytes (50X concentration, 90µL) Available	Cat. #	14-Plex Premixed Beads	23-Plex Premixed Beads
Anti-Rat Eotaxin Bead	2	✓	MXRETXN		✓
Anti-Rat GMCSF Bead	6	✓	MXRGM-CSF	✓	✓
Anti-Rat G-CSF Bead	7	✓	MXRG-CSF		✓
Anti-Rat IL-1α Bead	8	✓	MXRIL-1A	✓	✓
Anti-Rat MCP-1 Bead	13	✓	MXRMCP-1	✓	✓
Anti-Leptin Bead	16	✓	MXRME-LPTN		✓
Anti-Rat MIP-1α Bead	23	✓	MXRMIP-1A		✓
Anti-Rat IL-4 Bead	28	✓	MXRIL-4	✓	✓
Anti-Rat IL-1β Bead	34	✓	MXRIL-1B	✓	✓
Anti-Rat IL-2 Bead	36	✓	MXRIL-2	✓	✓
Anti-Rat IL-6 Bead	38	✓	MXRIL-6	✓	✓
Anti-Rat IL-13 Bead	45	✓	MXRIL-13		✓
Anti-Rat IL10 Bead	54	✓	MXRIL-10	✓	✓
Anti-Rat IL-12p70	56	✓	MXRIL-12	✓	✓
Anti-Rat IL-5 Bead	62	✓	MXRIL-5	✓	✓
Anti-Rat IFNγ Bead	64	✓	MXRIFN-G	✓	✓
Anti-Rat IL-17 Bead	67	✓	MXRIL-17		✓
Anti-Rat IL-18 Bead	69	✓	MXRIL-18	✓	✓
Anti-Rat IP-10 Bead	72	✓	MXRIP-10		✓
Anti-Rat GRO/KC Beads	73	✓	MXRGRO-KC	✓	✓
Anti-Rat RANTES Bead	75	✓	MXRRANTES		✓
Anti-Rat TNFα Bead	77	✓	MXRTNF-A	✓	✓
Anti-Rat VEGF Bead	99	✓	MXRVEGF		✓

MATERIALS REQUIRED BUT NOT PROVIDED

Reagents

1. Luminex Sheath Fluid (Luminex Catalogue #40-50000)

Instrumentation / Materials

1. Adjustable Pipettes with Tips capable of delivering 25 µL to 1000 µL
2. Multichannel Pipettes capable of delivering 5 µL to 50 µL or 25 µL to 200 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Rubber Bands
6. Absorbent Pads
7. Laboratory Vortex Mixer
8. Sonicator (Branson Ultrasonic Cleaner Model #B200 or equivalent)
9. Titer Plate Shaker (Lab-Line Instruments Model #4625 or equivalent)
10. Vacuum Filtration Unit (Millipore Vacuum Manifold Catalog #MSVMHTS00 or equivalent with Millipore Vacuum Pump Catalog #WP6111560 or equivalent)
11. Luminex 100™ IS, 200™, or HTS by Luminex Corporation
12. Plate Stand (Millipore Catalog # MX-STAND)

SAFETY PRECAUTIONS

- All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
- Sodium azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, sodium azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

TECHNICAL GUIDELINES

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- Do not use beyond the expiration date on the label.
- Do not mix or substitute reagents with those from other lots or sources.
- The Antibody-Immobilized Beads are light sensitive and must be protected from light at all times. Cover the assay plate containing beads with opaque plate lid or aluminum foil during all incubation steps.
- It is important to allow all reagents to warm to room temperature (20-25°C) before use in the assay.

- The bottom of the Microtiter Filter Plate should not be in direct contact with any surface during assay set-up or incubation times. The plate can be set on a plate stand or on the non-flat side of the plate cover or any other plate holder to raise the plate from the surface. A plate stand can be purchased separately from Millipore (Millipore Catalog #MX-STAND).
- Incomplete washing can adversely affect the assay outcome. All washing must be performed with the Wash Buffer provided.
- After the wash steps, keep the bottom of the Microtiter Filter Plate clean by blotting on paper towels or absorbent pads to prevent any leakage due to capillary action.
- Keep the vacuum suction on the plate as low as possible. It is recommended to have a vacuum setting that will remove 200 µL of buffer in $\geq$ 5 seconds (equivalent to < 100 mmHg).
- After hydration, all Standards and Controls must be transferred to polypropylene tubes.
- The Standards prepared by serial dilution must be used within 1 hour of preparation. Discard any unused standards except the standard stock which may be stored at $\leq$ -20°C for 1 month and at $\leq$ -80°C for greater than one month.
- If samples fall outside the dynamic range of the assay, further dilute the samples with the appropriate diluent and repeat the assay.
- Any unused mixed Antibody-Immobilized Beads may be stored in the Mixing Bottle at 2-8°C for up to one month.
- During the preparation of the standard curve, make certain to mix the higher concentration well before making the next dilution. Use a new tip with each dilution.
- The plate should be read immediately after the assay is finished. If, however, the plate cannot be read immediately, seal the plate, cover with aluminum foil or an opaque lid, and store the plate at 2-8°C for up to 24 hours. Prior to reading, agitate the plate on the plate shaker at room temperature for 10 minutes. Delay in reading a plate may result in decreased sensitivity for some cytokines and chemokines.
- The titer plate shaker should be set at a speed to provide maximum orbital mixing without splashing of liquid outside the wells. For the recommended plate shaker, this would be a setting of 5-7 which is approximately 500-800 rpm.
- Ensure that the needle probe is clean. This may be achieved by sonication and/or alcohol flushes. Adjust probe height according to the protocols recommended by Luminex to the kit filter plate using 3 alignment discs prior to reading an assay.
- For cell culture supernatants or tissue extraction, use the culture or extraction medium as the matrix solution in background, standard curve and control wells. If samples are diluted in Assay Buffer, use the Assay Buffer as matrix.
- For serum/plasma samples, use the Serum Matrix provided in the kit.
- For cell/tissue homogenate, the final cell or tissue homogenate should be prepared in a buffer that has a neutral pH, contains minimal detergents or strong denaturing detergents, and has an ionic strength close to physiological concentration. Avoid debris, lipids, and cell/tissue aggregates. Centrifuge samples before use.
- Vortex all reagents well before adding to plate.

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- It is recommended to centrifuge samples again at 3000xg for five minutes prior to assay setup.
- **Rat Serum should be diluted five-fold using the serum matrix as the sample diluent.** Dilute 1 part of rat serum with 4 parts Serum Matrix (e.g. add 12 µL rat serum to 48 µL of Serum Matrix for duplicate samples). Alternatively, at step at Step 8 in Section IX. Immunoassay Procedure, following addition of 20 µL of the Serum Matrix to each sample well, 5 µL undiluted serum sample can be added directly to sample wells. **Measurement of RANTES and GRO/KC in some serum sample expected to have high concentrations may require further dilutions (e.g. 1:20).**

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- EDTA is recommended as the anticoagulant in preparation of rat plasma samples.
- It is recommended to centrifuge samples again at 3000xg for five minutes prior to assay setup.
- **Rat Plasma should be diluted five-fold using the serum matrix as the sample diluent.** Dilute 1 part of rat plasma with 4 parts Serum Matrix (e.g. add 12 µL rat plasma to 48 µL of Serum Matrix for duplicate samples). Alternatively, at step at Step 8 in Section IX. Immunoassay Procedure, following addition of 20 µL of the Serum Matrix to each sample well, 5 µL undiluted plasma sample can be added directly to sample wells. **Measurement of RANTES and GRO/KC in some plasma samples expected to have high concentrations may require further dilutions (e.g. 1:20).**

C. Preparation of Tissue Culture Supernatant:

- Centrifuge the sample to remove debris and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple (>2) freeze/thaw cycles.
- Tissue culture supernatant may require a dilution with an appropriate control medium prior to assay.
- Tissue/cell extracts should be done in neutral buffers containing reagents and conditions that do not interfere with assay performance. Excess concentrations of detergent, salt, denaturants, high or low pH, etc. will negatively affect the assay. Organic solvents should be avoided. The tissue/cell extract samples should be free of particles such as cells or tissue debris.

NOTE:

- A maximum of 25 µL per well of diluted serum or plasma can be used. Tissue culture or other media may also be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.

PREPARATION OF REAGENTS FOR IMMUNOASSAY

A. Preparation of Antibody-Immobilized Beads

If premixed beads are used, sonicate the premixed bead bottle 30 seconds and then vortex for 1 minute before use.

For individual vials of beads, sonicate each antibody-bead vial for 30 seconds; vortex for 1 minute. Add 60 µL from each antibody bead vial to the Mixing Bottle and bring final volume to 3.0 mL with Bead Diluent. Vortex the mixed beads well. Unused portion may be stored at 2-8°C for up to one month.

Example 1: When using 7 cytokine antibody-immobilized beads, add 60 µL from each of the 7 bead sets to the Mixing Bottle. Then add 2.58 mL Bead Diluent.

Example 2: When using 20 cytokine antibody-immobilized beads, add 60 µL from each of the 20 bead sets to the Mixing Bottle. Then add 1.8 mL Bead Diluent.

B. Preparation of Quality Controls

Before use, reconstitute Quality Control 1 and Quality Control 2 with 250 µL deionized water. Invert the vial several times to mix and vortex. Allow the vial to sit for 5-10 minutes and then transfer the controls to appropriately labeled polypropylene microfuge tubes. Unused portion may be stored at ≤ -20°C for up to one month.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 30 mL of 10X Wash Buffer with 270 mL deionized water. Store unused portion at 2-8°C for up to one month.

D. Preparation of Serum Matrix

This step is required for serum or plasma samples only.

Add 1.0 mL deionized water and 4.0 mL Assay Buffer to the bottle containing lyophilized Serum Matrix. Mix well. Allow at least 10 minutes for complete reconstitution. Leftover reconstituted Serum Matrix should be stored at ≤ -20°C for up to one month.

E. Preparation of Rat Cytokine Standard

1.) Prior to use, reconstitute the Rat Cytokine Standard with 250 µL deionized water to give a 20,000 pg/mL concentration of standard for all analytes except Leptin, which has a 100,000 pg/mL concentration after reconstitution. Invert the vial several times to mix. Vortex the vial for 10 seconds. Allow the vial to sit for 5-10 minutes and then transfer the standard to an appropriately labeled polypropylene microfuge tube. This will be used as original standard; the unused portion may be stored at ≤ -20°C for up to one month.

2.) Preparation of Working Standards

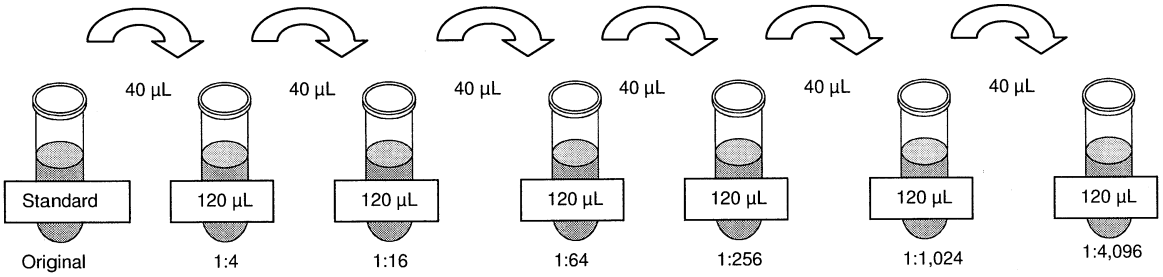
Label six polypropylene microfuge tubes 1:4, 1:16, 1:64, 1:256, 1:1,024 and 1:4,096. Add 120 µL of Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 40 µL of the original reconstituted standard to the 1:4 tube, mix well and transfer 40 µL of the 1:4 standard to the 1:16 tube, mix well and transfer 40 µL of the 1:16 standard to the 1:64 tube, mix well and transfer 40 µL of the 1:64 standard to 1:256 tube, mix well and transfer 40 µL of the 1:256 standard to the 1:1,024 tube and mix well, and transfer 40 µL of the 1:1,024 standard to the 1:4,096 tube and mix well. The 0 pg/mL standard (Background) will be Assay Buffer.

Based on individual needs, users may also use 3X or 5X serial dilutions for the standard curve.

Standard Concentration (pg/mL)	Volume of Deionized Water to Add	Volume of Standard to Add
Original	250 µL	0

Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
1:4	120 µL	40 µL of Original
1:16	120 µL	40 µL of 1:4
1:64	120 µL	40 µL of 1:16
1:256	120 µL	40 µL of 1:64
1:1,024	120 µL	40 µL of 1:256
1:4,096	120 µL	40 µL of 1:1,024

Standard Preparation:



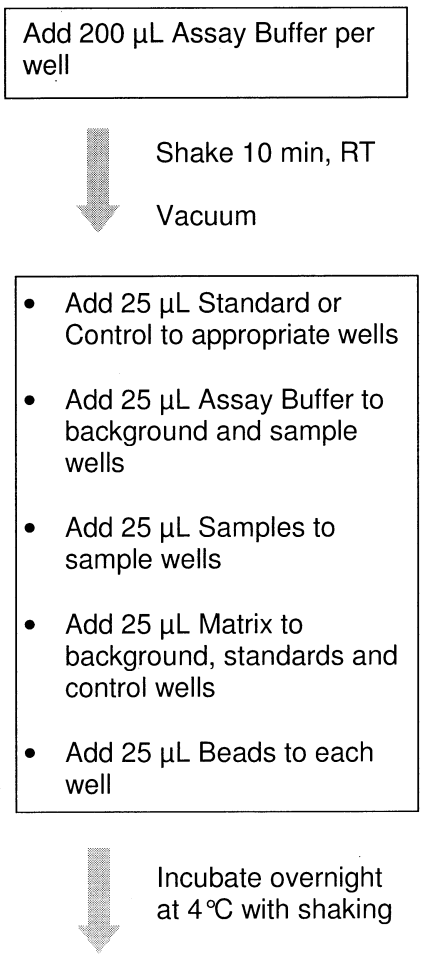
After serial dilutions, the tubes should have following concentrations for constructing standard curves.

Standard Tube (Dilution)	Concentrations for All Analytes Except for Leptin	Concentration for Leptin
Original	20,000 pg/mL	100,000 pg/mL
1:4	5,000 pg/mL	25,000 pg/mL
1:16	1,250 pg/mL	6,250 pg/mL
1:64	312.5 pg/mL	1,562.5 pg/mL
1:256	78.13 pg/mL	390.63 pg/mL
1:1,024	19.53 pg/mL	97.66 pg/mL
1:4,096	4.88 pg/mL	24.41 pg/mL

IMMUNOASSAY PROCEDURE

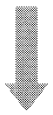
- Prior to beginning this assay, it is imperative to read this protocol completely and to thoroughly understand the Technical Guidelines.
- Allow all reagents to warm to room temperature (20-25°C) before use in the assay.
- Diagram the placement of Standards [0 (Background), 1:4096, 1:1024, 1:256, 1:64, 1:16, 1:4 and original], Controls 1 and 2, and Samples on Well Map Worksheet in a vertical configuration. (Note: Most instruments will only read the 96-well plate vertically by default.) It is recommended to run the assay in duplicate.
- Set the filter plate on a plate holder at all times during reagent dispensing and incubation steps so that the bottom of the plate does not touch any surface.

1. Prewet the filter plate by pipetting 200 µL of Assay Buffer into each well of the Microtiter Filter Plate. Seal and mix on a plate shaker for 10 minutes at room temperature (20-25°C).
2. Remove Assay Buffer by vacuum. **(NOTE: DO NOT INVERT PLATE.)** Blot excess Assay Buffer from the bottom of the plate with an absorbent pad or paper towels.
3. Add 25 µL of each Standard or Control into the appropriate wells. Assay Buffer should be used for the 0 pg/mL standard (Background).
4. Add 25 µL of Assay Buffer to the sample wells.
5. Add 25 µL of appropriate matrix solution to the background, standards, and control wells. When assaying serum or plasma, use the Serum Matrix provided in the kit. When assaying tissue culture medium, use identical control medium as the matrix solution. When assaying tissue/cell culture extract, use identical extraction buffer as the matrix solution.
6. Add 25 µL of diluted sample into the appropriate wells.
7. Vortex Mixing Bottle and add 25 µL of the Mixed or Premixed Beads to each well. (Note: During addition of beads, shake bead bottle intermittently to avoid settling.)
8. Seal the plate with a plate sealer, cover it with the lid. Wrap a rubber band around the plate holder, plate and lid and incubate with agitation on a plate shaker overnight (18-20 hours) at 4°C.



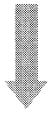
9. Gently remove fluid by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
10. Wash plate 2 times with 200 µL/well of Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Blot excess Wash Buffer from the bottom the plate by with an absorbent pad or paper towels.
11. Add 25 µL of Detection Antibodies into each well. (Note: Allow the Detection Antibodies to warm to room temperature prior to addition.)
12. Seal, cover with lid, and incubate with agitation on a plate shaker for 2 hours at room temperature (20-25°C). **DO NOT VACUUM AFTER INCUBATION.**
13. Add 25 µL Streptavidin-Phycoerythrin to each well containing the 25 µL of Detection Antibodies.
14. Seal, cover with lid and incubate with agitation on a plate shaker for 30 minutes at room temperature (20-25°C).
15. Gently remove all contents by vacuum. **(NOTE: DO NOT INVERT PLATE.)**
16. Wash plate 2 times with 200 µL/well Wash Buffer, removing Wash Buffer by vacuum filtration between each wash. Wipe any excess buffer on the bottom of the plate with a tissue.
17. Add 150 µL of Sheath Fluid to all wells. Resuspend the beads on a plate shaker for 5 minutes.
18. Run plate on Luminex 100™ IS, 200™, or HTS.
19. Save and analyze the Median Fluorescent Intensity (MFI) data using a weighted 5 or 4-parameter logistic or spline curve-fitting method for calculating cytokine/chemokines concentrations in samples.

    When calculating final sample concentrations, remember to multiply by the dilution factor.



Vacuum and wash 2X with 200 µL Wash Buffer

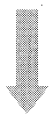
Add 25 µL Detection Antibody per well



Incubate 2 hours at RT

Do Not Vacuum

Add 25 µL Streptavidin-Phycoerythrin per well



Incubate for 30 minutes at RT

Vacuum and wash 2X with 200 µL Wash Buffer

Add 150 µL Sheath Fluid per well

Read on Luminex (100 µL, 50 beads per bead set)

EQUIPMENT SETTINGS

These specifications are for the Luminex 100™ IS v.1.7 or Luminex 100™ IS v2.1/2.2, Luminex 200™ v2.3, xPONENT, and Luminex HTS. Luminex instruments with other software (e.g. MasterPlex, StarStation, LiquiChip, Bio-Plex, LABScan100) would need to follow instrument instructions for gate settings and additional specifications from the vendors.

Events:	50, per bead		50, per bead		50, per bead	
Sample Size:	100 µL		100 µL		100 µL	
Gate Settings:	8,000 to 15,000					
Reporter Gain:	Default (Low PMT)					
Time Out:	60 seconds					
Bead Set:	14-Plex Premix Beads		23-Plex Premix Beads		Customizable 23-Plex Beads	
	rtGMCSF	6	rtEotaxin	2	rtEotaxin	2
	rtIL-1α	8	rtGMCSF	6	rtGMCSF	6
	rtMCP-1	13	rtGCSF	7	rtGCSF	7
	rtIL-4	28	rtIL-1α	8	rtIL-1α	8
	rtIL-1β	34	rtMCP-1	13	rtMCP-1	13
	rtIL-2	36	rtLeptin	16	rtLeptin	16
	rtIL-6	38	rtMIP-1α	23	rtMIP-1α	23
	rtIL-10	54	rtIL-4	28	rtIL-4	28
	rtIL-12p70	56	rtIL-1β	34	rtIL-1β	34
	rtIL-5	62	rtIL-2	36	rtIL-2	36
	rtIFNγ	64	rtIL-6	38	rtIL-6	38
	rtIL-18	69	rtIL-13	45	rtIL-13	45
	rtGRO/KC	73	rtIL-10	54	rtIL-10	54
	rtTNFα	77	rtIL-12p70	56	rtIL-12p70	56
			rtIL-5	62	rtIL-5	62
			rtIFNγ	64	rtIFNγ	64
			rtIL-17	67	rtIL-17	67
			rtIL-18	69	rtIL-18	69
			rtIP-10	72	rtIP-10	72
			rtGRO/KC	73	rtGRO/KC	73
			rtRANTES	75	rtRANTES	75
			rtTNFα	77	rtTNFα	77
			rtVEGF	99	rtVEGF	99

QUALITY CONTROLS

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert or can be located at the MILLIPORE website www.millipore.com/techlibrary/index.do using the catalog number as the keyword.

ASSAY CHARACTERISTICS

Assay Sensitivities (minimum detectable concentrations, pg/mL)

MinDC: Minimum Detectable Concentration is calculated by the StatLIA® Immunoassay Analysis Software from Brendan Technologies. It measures the true limits of detection for an assay by mathematically determining what the empirical MinDC would be if an infinite number of standard concentrations were run for the assay under the same conditions.

Cytokine	MinDC (pg/mL)
rtIL-1α	6.23
rtIL-1β	2.32
rtIL-2	3.67
rtIL-4	2.30
rtIL-5	2.89
rtIL-6	9.80
rtIL-10	5.41
rtIL-12p70	4.13
rtEotaxin	3.27
rtG-CSF	1.31
rtIP-10	3.78
rtLeptin	21.50

Cytokine	MinDC (pg/mL)
rtVEGF	4.93
rtIL-18	4.78
rtGMCSF	13.11
rtGRO/KC	2.06
rtIFNγ	4.88
rtMCP-1	3.81
rtTNFα	4.44
rtIL-13	23.2
rtIL-17	1.61
rtMIP-1α	1.94
rtRANTES	54.42

Precision

Intra-assay precision is generated from the mean of the %CV's from 8 reportable results across two different concentration of cytokines in one experiment. Inter-assay precision is generated from the mean of the %CV's from two reportable results each for two different concentrations of cytokine across 4 different experiments.

Cytokine	Intra-Assay Precision (%CV)	Interassay Precision (%CV)
rtIL-1α	5.80	10.2
rtIL-1β	7.53	12.4
rtIL-2	12.26	10.2
rtIL-4	5.93	8.8
rtIL-5	4.25	6.2
rtIL-6	10.37	14.3
rtIL-10	4.77	16.9
rtIL-12p70	6.53	11.2
rtIL-13	5.26	9.3
rtIL-17	5.96	5.9
rtIL-18	7.65	10.9
rtEotaxin	5.78	18.0
rtG-CSF	9.18	6.6
rtGMCSF	7.64	6.9
rtGRO/KC	7.39	14.1
rtIFNγ	5.91	14.7
rtIP-10	4.39	16.6
rtLeptin	8.15	8.5
rtMIP-1α	7.11	20.3
rtMCP-1	3.81	16.5
rtRANTES	8.34	17.7
rtTNFα	9.16	11.1
rtVEGF	9.16	8.3

Accuracy

Spike Recovery: The data represent mean percent recovery of 7 levels of spiked standards ranging from 31 to 2,000 pg/mL in serum matrices in 8 independent experiments.

Cytokine	Average % Recovery
rtIL-1α	89.97
rtIL-1β	77.97
rtIL-2	77.72
rtIL-4	88.92
rtIL-5	87.88
rtIL-6	89.63
rtIL-10	75.06
rtEotaxin	90.66
rtG-CSF	74.94
rtIP-10	91.35
rtLeptin	70.76

Cytokine	Average % Recovery
rtIL-12p70	94.71
rtIL-18	79.93
rtGMCSF	90.05
rtGRO/KC	78.63
rtIFNγ	87.59
rtMCP-1	76.30
rtTNFα	88.12
rtIL-13	83.26
rtIL-17	77.69
rtMIP-1 α	77.81
rtRANTES	71.67
rtVEGF	95.00

Cross-Reactivity

To test cross-reactivity among the assays in the panel, each cytokine was individually prepared at a concentration 5 times greater than the highest standard and tested as individual single-plex standards in the 14-plex assays using the multiplexed beads with immobilized capture antibodies and multiplexed detection antibody cocktail. There was none or negligible cross-reactivity among the antibodies with any of the other analytes in the panel.

TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Filter plate will not vacuum	Vacuum pressure is insufficient	Increase vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Samples have insoluble particles	Centrifuge samples just prior to assay set-up and use supernatant.
		If high lipid concentration, after centrifugation, remove lipid layer and use supernatant.
	Sample too viscous	May need to dilute sample.
Insufficient bead count	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds.
	Bead mix prepared incorrectly	Sonicate bead vials and vortex just prior to adding to bead mix bottle according to protocol. Agitate bead mix intermittently in reservoir while pipetting into the plate.
	Samples cause interference due to particulate matter or viscosity	See above. Also sample probe may need to be cleaned with alcohol flush, backflush and washes; or, if needed, probe should be removed and sonicated.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
Plate leaked	Vacuum pressure too high	Adjust vacuum pressure such that 0.2mL buffer can be suctioned in 3-5 seconds. May need to transfer contents to a new (prewetted) plate and continue.
	Plate set directly on table or absorbent towels during incubations or reagent additions	Set plate on plate stand or raised edge so bottom of filter is not touching any surface.
	Insufficient blotting of filter plate bottom causing wicking	Blot the bottom of the filter plate well with absorbent towels after each wash step.
	Pipette touching plate filter during additions	Pipette to the side of well.
	Probe height not adjusted correctly	Adjust probe to 3 alignment discs in well H6.
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using sealer appropriately and by pipetting with multichannel pipets without touching reagent in plate.
	Matrix used has endogenous analyte or interference	Check matrix ingredients for crossreacting components (e.g. interleukin modified tissue culture medium).
	Insufficient washes	Increase number of washes.

Beads not in region or gate	Luminex not calibrated correctly or recently	Calibrate Luminex based on instrument manufacturer's instructions at least once a week or if temperature has changed by >3°C.
	Gate settings not adjusted correctly	Some Luminex instruments (e.g. Bio-Plex) require different gate settings than those described in the kit protocol. Use instrument default settings.
	Wrong bead regions in protocol template	Check kit protocol for correct bead regions or analyte selection.
	Incorrect sample type used	Samples containing organic solvents or if highly viscous should be diluted or dialyzed as required.
	Instrument not washed or primed	Prime the Luminex 4 times to eliminate air bubbles. Wash 4 times with sheath fluid or water if there is any remnant alcohol or sanitizing liquid.
	Beads were exposed to light	Keep plate and bead mix covered with dark lid or aluminum foil during all incubation steps.
Signal for whole plate is same as background	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue.
	Streptavidin-Phycoerythrin was not added	Add Streptavidin-Phycoerythrin according to protocol. If Detection Antibody has already been vacuumed out, sensitivity may be low.
Low signal for standard curve	Detection Antibody may have been vacuumed out prior to adding Streptavidin Phycoerythrin	May need to repeat assay if desired sensitivity not achieved.
	Incubations done at incorrect temperatures, timings or agitation	Assay conditions need to be checked.
Signals too high, standard curves are saturated	Calibration target value set too high	With some Luminex instruments (e.g. Bio-Plex) default target setting for RP1 calibrator is set at High PMT. Use low target value for calibration and reanalyze plate.
	Plate incubation was too long with standard curve and samples	Use shorter incubation time.
Sample readings are out of range	Samples contain no or below detectable levels of analyte	If below detectable levels, it may be possible to use higher sample volume. Check with tech support for appropriate protocol modifications.
	Samples contain analyte concentrations higher than highest standard point	Samples may require dilution and reanalysis for that particular analyte.
	Standard curve was saturated at higher end of curve	See above.

High variation in samples and/or standards	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm all reagents are vacuumed out completely in all wash steps.
	Samples may have high particulate matter or other interfering substances	See above.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without splashing.
	Cross-well contamination	Check when reusing plate sealer that no reagent has touched sealer.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.

REPLACEMENT REAGENTS	**Catalog #**
Rat Cytokine/Chemokine Standard	LRC-8080
Rat Cytokine/Chemokine Quality Controls 1 & 2	LRC-6080
Serum Matrix	LMC-SD (optional)
Bead Diluent	LA-BD
Rat Cytokine Detection Antibodies	LRC-1080-1 LRC-1080-2 LRC-1080-3
Streptavidin-Phycoerythrin	L-SAPE4 L-SAPE6
Assay Buffer	L-MAB
Set of two 96-Well Filter Plates with Sealers	MX-PLATE
10X Wash Buffer	L-WB

Antibody-Immobilized Beads

Cytokine	Bead #	Cat. #
Eotaxin	2	MXRETXN
GMCSF	6	MXRGM-CSF
G-CSF	7	MXRG-CSF
RIL-1α	8	MXRIL-1A
MCP-1	13	MXRMCP-1
Leptin	16	MXRME-LPTN
MIP-1α	23	MXRMIP-1A
IL-4	28	MXRIL-4
IL-1β	34	MXRIL-1B
IL-2	36	MXRIL-2
IL-6	38	MXRIL-6
IL-13	45	MXRIL-13
IL-10	54	MXRIL-10

Cytokine	Bead #	Cat. #
IL-12p70	56	MXRIL-12
IL-5	62	MXRIL-5
IFNγ	64	MXRIFN-G
IL-17	67	MXRIL-17
IL-18	69	MXRIL-18
IP-10	72	MXRIP-10
GRO/KC	73	MXRGRO-KC
RANTES	75	MXRRANTES
TNF-α	77	MXRTNF-A
VEGF	99	MXRVEGF
Premixed 14-plex Beads		MXRCB-PMX
Premixed 23-plex Beads		MXRCB-PMX23

ORDERING INFORMATION

To place an order:

To assure the clarity of your custom cytokine kit order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits
- Selection of MILLIPLEX® Cytokine Analytes/Serum Matrix Requirements

FAX: (636) 441-8050
Toll-Free US: (800) MILLIPORE
Mail Orders: Millipore Corp.
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

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Conditions of Sale

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Material Safety Data Sheets (MSDS)

Material Safety Data Sheets for Millipore products may be ordered by fax or phone or through our website at www.millipore.com/techlibrary/index.do

WELL MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	0 pg/mL Standard (Background)	1:64 Standard	QC-I Control	Etc.								
B	0 pg/mL Standard (Background)	1:64 Standard	QC-I Control									
C	1:4,096 Standard	1:16 Standard	QC-II Control									
D	1:4,096 Standard	1:16 Standard	QC-II Control									
E	1:1,024 Standard	1:4 Standard	Sample 1									
F	1;1,024 Standard	1:4 Standard	Sample 1									
G	1:256 Standard	Original Standard	Sample 2									
H	1:256 Standard	Original Standard	Sample 2									

QUALITY CC ROL RANGES

Milliplex Rat Cytokine Kit
Catalog # RCYTO-80K

Milliplex Rat Cytokine Magnetic Panel
Catalog# RCYTOMAG-80K

Control Catalog #LRC-6080

Cytokine	QC Level	Lot #	Expected Range	Units	Cytokine	QC Level	Lot #	Expected Range	Units
Eotaxin	Control 1	RCYTO110	105 - 218	pg/mL	IL-13	Control 1	RCYTO110	130 - 270	pg/mL
	Control 2	RCYTO210	504 - 1046	pg/mL		Control 2	RCYTO210	609 - 1264	pg/mL
GM-CSF	Control 1	RCYTO110	276 - 573	pg/mL	IL-10	Control 1	RCYTO110	160 - 332	pg/mL
	Control 2	RCYTO210	756 - 1570	pg/mL		Control 2	RCYTO210	660 - 1372	pg/mL
G-CSF	Control 1	RCYTO110	187 - 388	pg/mL	IL-12p70	Control 1	RCYTO110	124 - 257	pg/mL
	Control 2	RCYTO210	825 - 1714	pg/mL		Control 2	RCYTO210	477 - 990	pg/mL
IL-1A	Control 1	RCYTO110	156 - 323	pg/mL	IL-5	Control 1	RCYTO110	99 - 205	pg/mL
	Control 2	RCYTO210	575 - 1194	pg/mL		Control 2	RCYTO210	474 - 985	pg/mL
MCP-1	Control 1	RCYTO110	96 - 199	pg/mL	IFN-G	Control 1	RCYTO110	117 - 244	pg/mL
	Control 2	RCYTO210	475 - 986	pg/mL		Control 2	RCYTO210	552 - 1146	pg/mL
LEPTIN	Control 1	RCYTO110	829 - 1722	pg/mL	IL-17	Control 1	RCYTO110	146 - 304	pg/mL
	Control 2	RCYTO210	3574 - 7423	pg/mL		Control 2	RCYTO210	696 - 1445	pg/mL
MIP-1A	Control 1	RCYTO110	125 - 259	pg/mL	IL-18	Control 1	RCYTO110	123 - 255	pg/mL
	Control 2	RCYTO210	495 - 1027	pg/mL		Control 2	RCYTO210	592 - 1230	pg/mL
IL-4	Control 1	RCYTO110	139 - 289	pg/mL	IP-10	Control 1	RCYTO110	136 - 283	pg/mL
	Control 2	RCYTO210	623 - 1294	pg/mL		Control 2	RCYTO210	693 - 1439	pg/mL
IL-1B	Control 1	RCYTO110	154 - 319	pg/mL	GRO-KC	Control 1	RCYTO110	128 - 265	pg/mL
	Control 2	RCYTO210	702 - 1458	pg/mL		Control 2	RCYTO210	627 - 1302	pg/mL
IL-2	Control 1	RCYTO110	167 - 346	pg/mL	RANTES	Control 1	RCYTO110	146 - 303	pg/mL
	Control 2	RCYTO210	672 - 1395	pg/mL		Control 2	RCYTO210	587 - 1219	pg/mL
IL-6	Control 1	RCYTO110	156 - 325	pg/mL	TNF-A	Control 1	RCYTO110	148 - 307	pg/mL
	Control 2	RCYTO210	635 - 1320	pg/mL		Control 2	RCYTO210	759 - 1577	pg/mL
					VEGF	Control 1	RCYTO110	130 - 269	pg/mL
						Control 2	RCYTO210	600 - 1245	pg/mL

Note: The Quality Control Ranges are generated with overnight assay format using serum matrix provided in the kit. Quality Control values in culture media are not tested.

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Cytokine/Chemokine Analysis of Oral Cavity and Serum: Quality Control Data

	Cytokine/Chemokine (pg/mL)										
Sample	**Eotaxin**	**G-CSF**	**GM-CSF**	**GRO/KC**	**IFN-γ**	**IL-10**	**IL-12p70**	**IL-13**	**IL-17**	**IL-18**	**IL-1a**
Control 1	222	295	191	201	149	189	177	214	222	176	170
Expect. Range	**105-218**	**187-388**	**276-573**	**128-265**	**117-244**	**160-332**	**124-257**	**130-270**	**146-304**	**123-255**	**156-323**
Control 2	841	1201	958	1042	484	864	777	974	1042	884	822
Expect. Range	**504-1046**	**825-1714**	**756-1570**	**627-1302**	**552-1146**	**660-1372**	**477-990**	**609-1264**	**696-1445**	**592-1230**	**575-1194**

	Cytokine/Chemokine (pg/mL)											
Sample	**IL-1b**	**IL-2**	**IL-4**	**IL-5**	**IL-6**	**IP-10**	**Leptin**	**MCP-1[a]**	**MIP-1a**	**RANTES**	**TNF-a**	**VEGF**
Control 1	224	184	199	172	88	315	1345	136	183	212	173	200
Expect. Range	**154-319**	**167-346**	**139-289**	**99-205**	**156-325**	**136-283**	**829-1722**	**96-199**	**125-259**	**146-303**	**148-307**	**130-269**
Control 2	933	910	1013	762	372	1115	6155	614	654	750	889	840
Expect. Range	**702-1458**	**672-1395**	**623-1294**	**474-985**	**635-1320**	**693-1439**	**3574-7423**	**475-986**	**495-1027**	**587-1219**	**759-1577**	**600-1245**

[a]MCP-1 analysis performed using 4 parameter logistic curve, instead of 5 parameter logistic curve.

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Cytokine/Chemokine Analysis of Duodenum: Quality Control Data

	Cytokine/Chemokine (pg/mL)										
Sample	**Eotaxin**	**G-CSF**	**GM-CSF**	**GRO/KC**	**IFN-γ**	**IL-10**	**IL-12p70**	**IL-13**	**IL-17**	**IL-18**	**IL-1a**
Control 1	222	295	191	201	149	189	177	214	222	176	170
Expect. Range	**105-218**	**187-388**	**276-573**	**128-265**	**117-244**	**160-332**	**124-257**	**130-270**	**146-304**	**123-255**	**156-323**
Control 2	841	1201	958	1042	484	864	777	974	1042	884	822
Expect. Range	**504-1046**	**825-1714**	**756-1570**	**627-1302**	**552-1146**	**660-1372**	**477-990**	**609-1264**	**696-1445**	**592-1230**	**575-1194**

	Cytokine/Chemokine (pg/mL)											
Sample	**IL-1b**	**IL-2**	**IL-4**	**IL-5**	**IL-6**	**IP-10**	**Leptin**	**MCP-1[a]**	**MIP-1a**	**RANTES**	**TNF-a**	**VEGF**
Control 1	224	184	199	172	88	315	1345	136	183	212	173	200
Expect. Range	**154-319**	**167-346**	**139-289**	**99-205**	**156-325**	**136-283**	**829-1722**	**96-199**	**125-259**	**146-303**	**148-307**	**130-269**
Control 2	933	910	1013	762	372	1115	6155	614	654	750	889	840
Expect. Range	**702-1458**	**672-1395**	**623-1294**	**474-985**	**635-1320**	**693-1439**	**3574-7423**	**475-986**	**495-1027**	**587-1219**	**759-1577**	**600-1245**

[a]MCP-1 analysis performed using 4 parameter logistic curve, instead of 5 parameter logistic curve.

Appendix H

Pathology Contributing Scientist Report

Pathology Contributing Scientist Report for the

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Submitted by:

Jill F. Mann, D.V.M., D.A.C.V.P.
Advanced Research Pathologist
Southern Research Institute
Birmingham, Alabama

Southern Research Study Number: 13026.01.02

June 15, 2011

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1.0 SIGNATURE PAGE

Pathology Contributing Scientist Report for the

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Jill F Mann 6/15/11

Jill F. Mann, D.V.M., D.A.C.V.P. Date
Advanced Research Pathologist

2.0 SUMMARY

The objective of this study was to evaluate the toxicity and potential mechanisms of action of sodium dichromate dihydrate (SDD) administered in drinking water to rats for 90 days. Samples were collected for various types of tests including gross and histopathologic evaluation of tissues from 30 rats (Day 8) and 60 rats (Day 91) that received 0, 0.3, 4, 60, 170, or 520 mg/L SDD orally. No SDD-related macroscopic lesions were observed on Day 8 or Day 91. The small intestine (duodenum and jejunum), oral mucosa, and gross lesions were examined microscopically. On Day 8, SDD-related microscopic lesions were observed in the small intestine (duodenum or jejunum). In the duodenum, test article-related microscopic lesions included histiocytic cellular infiltration (170 and 520 mg/L), apoptosis (60, 170, and 520 mg/L), villous atrophy (170 and 520 mg/L), and crypt hyperplasia (60, 170, and 520 mg/L). In the jejunum, test article-related microscopic lesions included apoptosis (170 and 520 mg/L), villous atrophy (170 and 520 mg/L), and crypt hyperplasia (170 and 520 mg/L). On Day 91, SDD-related microscopic lesions were observed in the small intestine (duodenum or jejunum). In the duodenum, test article-related microscopic lesions included histiocytic cellular infiltration (60, 170, and 520 mg/L), apoptosis (60, 170, and 520 mg/L), villous atrophy (170 mg/L), and crypt hyperplasia (170 and 520 mg/L). In the jejunum, test article-related microscopic lesions included histiocytic cellular infiltration (60, 170, and 520 mg/L), apoptosis (170 mg/L), and crypt hyperplasia (170 or 520 mg/L). No microscopic lesions were observed in the oral mucosa on Day 8 and Day 91.

3.0 METHODS AND MATERIALS

The test article, sodium dichromate dihydrate (SDD), was given orally to a total of 90 female Fischer rats at dose levels of 0 (tap water or “vehicle control”), 0.3, 4, 60, 170, or 520 mg/L in drinking water. Gross necropsy and tissue collection for histopathologic evaluation were performed on 5 rats/group on Day 8 and 10 rats/group on Day 91. A complete set of tissues as defined in the study protocol was evaluated microscopically and assigned morphologic diagnoses including topographic qualifiers where indicated. Microscopic lesions were graded, with few exceptions, using a numerical scoring system in which 1 = minimal, 2 = mild, 3 = moderate, and 4 = marked. Specific grading of individual lesions is discussed in the Results section.

4.0 RESULTS

Macroscopic Observations

Individual animal macroscopic observations are presented in Table H1 and are summarized in Table H2.

Day 8

On Day 8 no SDD-related macroscopic lesions were observed.

Day 91

On Day 91 no SDD-related macroscopic lesions were observed.

Microscopic Observations

Individual animal microscopic observations are presented in Table H3 and are summarized in Table H4.

Test article-related microscopic lesions observed in the small intestine included histiocytic cellular infiltration, apoptosis, crypt hyperplasia, and villous atrophy. Histiocytic cellular infiltration (Figures H1 and H2) consisted of the presence of large histiocytes (macrophages) with intracytoplasmic pale eosinophilic globular material in the lamina propria of the villous tips. Histiocytic cellular infiltration was graded based upon the following criteria: Minimal = occasional histiocyte observed; mild = small clusters of histiocytes which were readily observed using the 10× objective and present in $\geq$ 50% and $\leq$ 75% of villi; moderate = large clusters of histiocytes which were readily observed using the 10× objective and present in $\geq$ 75% and $\leq$ 90% of villi; marked = large clusters of histiocytes which were readily observed using the 10× objective and present in $\geq$ 90% of villi. Apoptosis (Figure H2) consisted of the presence of apoptotic bodies in the lamina propria or villous tip epithelial cells. Apoptosis was graded based on the following criteria: Minimal = $<$ 5 apoptotic cells per 40× or high power field (HPF); mild = approximately $\geq$ 5 and $\leq$ 10 apoptotic cells per HPF; moderate = $\geq$ 10 and $\leq$ 20 apoptotic cells per HPF; marked = $\geq$ 20 apoptotic cells per HPF. Crypt hyperplasia (Figures H5 and H6) consisted of increased numbers of crypt epithelial cells in the small intestine. The hyperplastic crypt epithelium displayed increased cytoplasmic basophilia. The crypt hyperplasia was diffuse

in distribution and most or all crypts appeared to have been involved on each affected tissue section examined. Crypt hyperplasia was graded based upon the following criteria: Minimal = ≥ 1 and ≤ 2 times the normal crypt depth; mild = ≥ 2 and ≤ 3 times the normal crypt depth; moderate = ≥ 3 and ≤ 4 times the normal crypt depth; marked = ≥ 4 times the normal crypt depth. Villous atrophy (Figure H5) consisted of shortened villi which were occasionally blunted and fused. The villous atrophy was diffuse in distribution and most or all villi appeared to have been involved on each affected tissue section examined. Villous atrophy was graded based upon the following criteria: Minimal = < 25% decrease in villous height; mild = ≥ 25% and ≤ 50% decrease in villous height; moderate = ≥ 50% and ≤ 75% decrease in villous height; marked = ≥ 75% decrease in villous height. Figures H3 and H4 were included to provide examples of small intestine from a control animal.

Day 8

On Day 8, SDD-related microscopic lesions were observed in the small intestine (duodenum or jejunum). In the duodenum, test article-related microscopic lesions included minimal to mild histiocytic cellular infiltration, minimal to mild apoptosis, minimal to mild villous atrophy, and minimal to moderate crypt hyperplasia. The level at which microscopic lesions were not observed in the duodenum on Day 8 was 4 mg/L. In the jejunum, test article-related microscopic lesions included minimal to mild apoptosis, minimal villous atrophy, and minimal, mild, or moderate crypt hyperplasia. The level at which microscopic lesions were not observed in the jejunum on Day 8 was 60 mg/L.

No microscopic lesions were observed in the oral mucosa on Day 8.

Day 91

On Day 91, SDD-related microscopic lesions were observed in the small intestine (duodenum or jejunum). In the duodenum, test article-related microscopic lesions included minimal to marked histiocytic cellular infiltration, minimal to mild apoptosis, mild villous atrophy, and minimal crypt hyperplasia. The level at which microscopic lesions were not observed in the duodenum on Day 91 was 4 mg/L. In the jejunum, test article-related microscopic lesions included minimal to mild histiocytic cellular infiltration, minimal apoptosis, moderate villous atrophy, and minimal

crypt hyperplasia. The level at which test article-related microscopic lesions were not observed in the jejunum on Day 91 was 4 mg/L.

Microscopic lesions observed in the jejunum of one animal in the 4 mg/L dose group (3F163) necropsied on Day 91 were similar to the SDD-related lesions (Figures H7 and H8), but may have been unique to this animal and not test article related. Lesions observed in this animal included a moderate depletion of crypts with replacement by fibrous connective tissue (moderate fibrosis) and mild chronic inflammation. Animal 3F163 also had moderate villous atrophy and minimal apoptosis which may have actually been related to the crypt depletion, fibrosis, and inflammation and not necessarily to the administration of SDD.

Minimal mineralization was observed in the mucosal associated lymphoid tissue (Peyer's patch) of the jejunum in three SDD-treated animals (0.3, 4, and 60 mg/L). Mineralization is occasionally observed in the Peyer's patches of rats and may be secondary to inflammation and necrosis.[(1)] Two of the SDD-treated animals (0.3 and 60 mg/L) had no other microscopic lesions in the jejunum. One SDD-treated animal (4 mg/L) had minimal crypt hyperplasia in addition to the mineralization. Mineralization of the Peyer's patches in these three SDD-treated rats was considered to have been a spontaneous and incidental event and not directly related to the administration of SDD.

On Day 91, additional microscopic lesions were observed in the liver (hepatodiagphragmatic nodule), mesentery (fat necrosis), and skin (inflammation and hair follicle atrophy). These lesions were considered to have been spontaneous and incidental events and not related to the administration of SDD.

No microscopic lesions were observed in the oral mucosa on Day 91.

5.0 DISCUSSION AND CONCLUSIONS

The objective of this study was to evaluate the toxicity and potential mechanisms of action of sodium dichromate dihydrate (SDD) administered in drinking water to rats for 90 days. Samples were collected for various tests including gross and histopathologic evaluation of the small

intestine (duodenum and jejunum) and the oral mucosa. In general, microscopic lesions were observed more frequently in the duodenum than the jejunum on Day 8 and Day 91. Crypt hyperplasia and villous atrophy were observed with higher frequency and increased severity in the duodenum and jejunum of animals necropsied on Day 8 than those lesions observed on Day 91. These findings are consistent with the mechanisms of repair following an injury to the intestinal mucosa.[(2)] After an injury, the damaged cells are shed from the villous tips in about 2-8 days. If the injury is minor, the lost cells are replaced by the lateral migration of adjacent intact epithelial cells very quickly. More severe injury requires hyperplasia and differentiation of stem cells that are present in the crypt epithelium. These hyperplastic cells with increased mitoses and increased cytoplasmic basophilia will migrate up the villous to replace lost cells. In general the duodenum will require longer time to repair due to the fact that the villi are longer than villi present in more distal sections of the small intestine. If the crypt epithelium is unable to adequately replace the villous tip epithelium then stromal collapse with blunting and fusion of villi can occur. Histiocytic cellular infiltration was observed with much greater frequency and severity in the duodenum and jejunum of animals necropsied on Day 91 than those lesions observed on Day 8. Apoptosis was observed with increased frequency on Day 8 than on Day 91. The microscopic lesions that were observed in the current study were consistent with the type of non-neoplastic lesions that were observed in previous studies in which SDD was administered to rats (histiocytic cellular infiltration) or mice (histiocytic cellular infiltration, diffuse epithelial hyperplasia) for 2 years.[(3)] In the previous studies, histiocytic cellular infiltration was observed in the duodenum of rats and in the duodenum and jejunum of mice and diffuse or focal epithelial hyperplasia was observed in the duodenum and jejunum of mice.[(3)] In the current rat study, the intestinal lesions were more similar to lesions observed in the previous mouse study than to the previous rat study. The reason for this discrepancy in the types of intestinal lesions observed in the current rat study versus the previous study is unknown.

In conclusion, SDD-related microscopic lesions observed in the small intestine included histiocytic cellular infiltration, apoptosis, crypt hyperplasia, and villous atrophy. In general, the duodenum was affected more severely than the jejunum. Crypt hyperplasia and villous atrophy were observed more frequently on Day 8 while histiocytic cellular infiltration was observed more frequently on Day 91.

6.0 REFERENCES

1. Kuper, C. F., "Histopathology of Mucosal-Associated Lymphoid Tissue" *Toxicologic Pathology*, 34:609-615, 2006.
2. Maxie, M. G. , Pathology of Domestic Animals Vol 2. Elsevier Saunders. New York 2007. Pp 76-77.
3. NTP (2008). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N rats and B6C3F1 mice (drinking water studies), NTP TR 546. NIH Publication No. 08-5887.

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 8

SEX: FEMALE						
	GROUP	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	.	.	.	.	.
	NUMBER	7	7	7	7	7
		1	2	3	4	5
ANIMAL IDENTIFICATION;		+	+	+	+	+
Submitted		P	P	P	P	P
ESOPHAGUS;		N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
LIVER;		N	N	N	N	N
LYMPH NODE, MESENTERIC;		N	N	N	N	N
ORAL MUCOSA;		N	N	N	N	N
STOMACH;		N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 8

SEX: FEMALE	GROUP	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	1	1	1	1	1
	NUMBER	4	4	4	4	5
		6	7	8	9	0
ANIMAL IDENTIFICATION;		+	+	+	+	+
Submitted		P	P	P	P	P
ESOPHAGUS;		N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
LIVER;		N	N	N	N	N
LYMPH NODE, MESENTERIC;		N	N	N	N	N
ORAL MUCOSA;		N	N	N	N	N
STOMACH;		N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 8

SEX: FEMALE	GROUP	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	2	2	2	2	2
	NUMBER	2	2	2	2	2
		1	2	3	4	5
ANIMAL IDENTIFICATION;		+	+	+	+	+
Submitted		P	P	P	P	P
ESOPHAGUS;		.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
LIVER;		.	.	.	.	.
LYMPH NODE, MESENTERIC;		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
STOMACH;		.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 8

SEX: FEMALE						
	GROUP	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	2	2	2	2	3
	NUMBER	9	9	9	9	0
		6	7	8	9	0
ANIMAL IDENTIFICATION;		+	+	+	+	+
Submitted		P	P	P	P	P
ESOPHAGUS;		N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
LIVER;		N	N	N	N	N
LYMPH NODE, MESENTERIC;		N	N	N	N	N
ORAL MUCOSA;		N	N	N	N	N
STOMACH;		N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 8

SEX: FEMALE	GROUP	5	5	5	5	5
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	3	3	3	3	3
	NUMBER	7	7	7	7	7
		1	2	3	4	5
ANIMAL IDENTIFICATION;		+	+	+	+	+
Submitted		P	P	P	P	P
ESOPHAGUS;		.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
LIVER;		.	.	.	.	.
LYMPH NODE, MESENTERIC;		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
STOMACH;		.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 8

SEX: FEMALE	GROUP	6	6	6	6	6
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	4	4	4	4	4
	NUMBER	4	4	4	4	5
		6	7	8	9	0
ANIMAL IDENTIFICATION;		+	+	+	+	+
Submitted		P	P	P	P	P
ESOPHAGUS;		N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
LIVER;		N	N	N	N	N
LYMPH NODE, MESENTERIC;		N	N	N	N	N
ORAL MUCOSA;		N	N	N	N	N
STOMACH;		N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	1	1	1	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	.	.	.	.	.	.	.	.
	NUMBER	1	1	1	1	1	1	1	1
		1	2	3	4	5	6	7	8
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	.	.	.	.	.	.
BONE MARROW SMEAR;		.	.	.	.	.	+	+	+
Submitted		.	.	.	.	.	P	P	P
ESOPHAGUS;		N	N	N	N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N	N	N	N
LIVER;		N	N	N	N	N	N	N	N
medial lobe; Hernia		.	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	N	N	N	N	N	N
MESENTERY;		.	.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE								
GROUP	1	1	1	1	1	1	1	1
REMOVAL REASON	S	S	S	S	S	S	S	S
ANIMAL	.	.	.	.	.	.	.	.
NUMBER	1	1	1	1	1	1	1	1
	1	2	3	4	5	6	7	8
SKIN;	.	.	.	.	.	.	.	.
Discoloration; red; bilateral	.	.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation	.	.	.	.	.	.	.	.
face; Alopecia	.	.	.	.	.	.	.	.
face; Discoloration; red; bilateral	.	.	.	.	.	.	.	.
face; Discoloration; red; left	.	.	.	.	.	.	.	.
vulva; Discoloration; brown	.	.	.	.	.	.	.	.
STOMACH;	N	N	N	N	N	N	N	N
UTERUS;	.	.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral	.	.	.	.	.	.	.	.
horn; Dilatation; bilateral	.	.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral	.	.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	1	1	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	.	.	.	.	.	.	.
	NUMBER	1 9	2 0	5 1	5 2	5 3	5 4	5 5
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	N	N	N	N	N
BONE MARROW SMEAR;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
ESOPHAGUS;		N	N	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	.	.	.	.	.
LIVER;		N	N	.	.	.	.	.
medial lobe; Hernia		.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	.	.	.	.	.
MESENTERY;		.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	1	1	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	.	.	.	.	.	.	.
	NUMBER	1 9	2 0	5 1	5 2	5 3	5 4	5 5
SKIN;		.	.	.	.	.	.	.
Discoloration; red; bilateral		.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation		.	.	.	.	.	.	.
face; Alopecia		.	.	.	.	.	.	.
face; Discoloration; red; bilateral		.	.	.	.	.	.	.
face; Discoloration; red; left		.	.	.	.	.	.	.
vulva; Discoloration; brown		.	.	.	.	.	.	.
STOMACH;		N	N	.	.	.	.	.
UTERUS;		.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral		.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	2	2	2	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	.	.	.	.	.	.	.	.
	NUMBER	8 6	8 7	8 8	8 9	9 0	9 1	9 2	9 3
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	.	.	.	.	.	.
BONE MARROW SMEAR;		.	.	.	.	.	+	+	+
Submitted		.	.	.	.	.	P	P	P
ESOPHAGUS;		N	N	N	N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N	N	N	N
LIVER;		N	N	N	N	N	N	N	N
medial lobe; Hernia		.	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	N	N	N	N	N	N
MESENTERY;		.	.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	2	2	2	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	.	.	.	.	.	.	.	.
	NUMBER	8	8	8	8	9	9	9	9
		6	7	8	9	0	1	2	3
SKIN;		.	.	.	.	.	.	.	.
Discoloration; red; bilateral		.	.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation		.	.	.	.	.	.	.	.
face; Alopecia		.	.	.	.	.	.	.	.
face; Discoloration; red; bilateral		.	.	.	.	.	.	.	.
face; Discoloration; red; left		.	.	.	.	.	.	.	.
vulva; Discoloration; brown		.	.	.	.	.	.	.	.
STOMACH;		N	N	N	N	N	N	N	N
UTERUS;		.	.	.	.	.	.	+	.
lumen; horn; Dilatation; bilateral		.	.	.	.	.	.	.	.
horn; Dilatation; bilateral		.	.	.	.	.	.	P	.
horn; lumen; Dilatation; bilateral		.	.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	2	2	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	.	.	1	1	1	1	1
	NUMBER	9	9	2	2	2	2	3
		4	5	6	7	8	9	0
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	N	N	N	N	N
BONE MARROW SMEAR;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
ESOPHAGUS;		N	N	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	.	.	.	.	.
LIVER;		N	N	.	.	.	.	.
medial lobe; Hernia		.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	.	.	.	.	.
MESENTERY;		.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	2	2	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	.	.	1	1	1	1	1
	NUMBER	9	9	2	2	2	2	3
		4	5	6	7	8	9	0
SKIN;		.	.	.	.	.	.	.
Discoloration; red; bilateral		.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation		.	.	.	.	.	.	.
face; Alopecia		.	.	.	.	.	.	.
face; Discoloration; red; bilateral		.	.	.	.	.	.	.
face; Discoloration; red; left		.	.	.	.	.	.	.
vulva; Discoloration; brown		.	.	.	.	.	.	.
STOMACH;		N	N	.	.	.	.	.
UTERUS;		.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral		.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	3	3	3	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	1	1	1	1	1	1	1	1
	NUMBER	6	6	6	6	6	6	6	6
		1	2	3	4	5	6	7	8
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	.	.	.	.	.	.
BONE MARROW SMEAR;		.	.	.	.	.	+	+	+
Submitted		.	.	.	.	.	P	P	P
ESOPHAGUS;		.	.	.	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	N	N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N	N	N	N
LIVER;		.	.	.	.	.	.	.	.
medial lobe; Hernia		.	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		.	.	.	.	.	.	.	.
MESENTERY;		.	+	.	.	.	.	+	.
fat; Mass; mottled		.	.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.	.
fat; Mass; yellow		.	P	.	.	.	.	P	.
fat; Nodule; mottled		.	.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE								
GROUP	3	3	3	3	3	3	3	3
REMOVAL REASON	S	S	S	S	S	S	S	S
ANIMAL	1	1	1	1	1	1	1	1
NUMBER	6	6	6	6	6	6	6	6
	1	2	3	4	5	6	7	8
SKIN;	.	.	+	.	.	.	.	.
Discoloration; red; bilateral	.	.	P	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation	.	.	.	.	.	.	.	.
face; Alopecia	.	.	.	.	.	.	.	.
face; Discoloration; red; bilateral	.	.	.	.	.	.	.	.
face; Discoloration; red; left	.	.	.	.	.	.	.	.
vulva; Discoloration; brown	.	.	.	.	.	.	.	.
STOMACH;	.	.	.	.	.	.	.	.
UTERUS;	.	.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral	.	.	.	.	.	.	.	.
horn; Dilatation; bilateral	.	.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral	.	.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	3	3	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	1	1	2	2	2	2	2
	NUMBER	6	7	0	0	0	0	0
		9	0	1	2	3	4	5
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	N	N	N	N	N
BONE MARROW SMEAR;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
ESOPHAGUS;		.	.	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	.	.	.	.	.
LIVER;		.	.	.	.	.	.	.
medial lobe; Hernia		.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		.	.	.	.	.	.	.
MESENTERY;		.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	3	3	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	1	1	2	2	2	2	2
	NUMBER	6	7	0	0	0	0	0
		9	0	1	2	3	4	5
SKIN;		.	.	.	.	.	.	.
Discoloration; red; bilateral		.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation		.	.	.	.	.	.	.
face; Alopecia		.	.	.	.	.	.	.
face; Discoloration; red; bilateral		.	.	.	.	.	.	.
face; Discoloration; red; left		.	.	.	.	.	.	.
vulva; Discoloration; brown		.	.	.	.	.	.	.
STOMACH;		.	.	.	.	.	.	.
UTERUS;		.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral		.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	4	4	4	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	2	2	2	2	2	2	2	2
	NUMBER	3	3	3	3	4	4	4	4
		6	7	8	9	0	1	2	3
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	.	.	.	.	.	.
BONE MARROW SMEAR;		.	.	.	.	.	+	+	+
Submitted		.	.	.	.	.	P	P	P
ESOPHAGUS;		N	N	N	N	N	N	N	N
SMALL INTESTINE, DUODENUM;		N	N	N	N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N	N	N	N
LIVER;		N	N	N	N	N	N	N	N
medial lobe; Hernia		.	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	N	N	N	N	N	N
MESENTERY;		.	.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	4	4	4	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	2	2	2	2	2	2	2	2
	NUMBER	3	3	3	3	4	4	4	4
		6	7	8	9	0	1	2	3
SKIN;		.	.	.	.	.	.	.	.
Discoloration; red; bilateral		.	.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation		.	.	.	.	.	.	.	.
face; Alopecia		.	.	.	.	.	.	.	.
face; Discoloration; red; bilateral		.	.	.	.	.	.	.	.
face; Discoloration; red; left		.	.	.	.	.	.	.	.
vulva; Discoloration; brown		.	.	.	.	.	.	.	.
STOMACH;		N	N	N	N	N	N	N	N
UTERUS;		.	.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral		.	.	.	.	.	.	.	.
horn; Dilatation; bilateral		.	.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral		.	.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	4	4	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	2	2	2	2	2	2	2
	NUMBER	4	4	7	7	7	7	8
		4	5	6	7	8	9	0
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	N	N	N	N	N
BONE MARROW SMEAR;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
ESOPHAGUS;		N	N	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	.	.	.	.	.
LIVER;		+	N	.	.	.	.	.
medial lobe; Hernia		P	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	.	.	.	.	.
MESENTERY;		.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	4	4	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	2	2	2	2	2	2	2
	NUMBER	4	4	7	7	7	7	8
		4	5	6	7	8	9	0
SKIN;		.	.	.	.	.	.	.
Discoloration; red; bilateral		.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation		.	.	.	.	.	.	.
face; Alopecia		.	.	.	.	.	.	.
face; Discoloration; red; bilateral		.	.	.	.	.	.	.
face; Discoloration; red; left		.	.	.	.	.	.	.
vulva; Discoloration; brown		.	.	.	.	.	.	.
STOMACH;		N	N	.	.	.	.	.
UTERUS;		.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; Dilatation; bilateral		.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral		.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	5	5	5	5	5	5	5	5
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	3	3	3	3	3	3	3	3
	NUMBER	1	1	1	1	1	1	1	1
		1	2	3	4	5	6	7	8
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	.	.	.	.	.	.
BONE MARROW SMEAR;		.	.	.	.	.	+	+	+
Submitted		.	.	.	.	.	P	P	P
ESOPHAGUS;		.	.	.	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	N	N	N	N	N	N
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N	N	N	N
LIVER;		.	.	.	.	.	.	.	.
medial lobe; Hernia		.	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		.	.	.	.	.	.	.	.
MESENTERY;		+	.	.	.	.	+	.	+
fat; Mass; mottled		.	.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.	P
fat; Mass; yellow		P	.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	P	.	.
ORAL MUCOSA;		N	N	N	N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	5	5	5	5	5	5	5	5
	REMOVAL REASON	S	S	S	S	S	S	S	S
	ANIMAL	3	3	3	3	3	3	3	3
	NUMBER	1	1	1	1	1	1	1	1
		1	2	3	4	5	6	7	8
SKIN;			+	+	+	+	+	+	+
Discoloration; red; bilateral			.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation			.	.	.	P	.	P	.
face; Alopecia			P	P	P	.	P	.	P
face; Discoloration; red; bilateral			.	.	.	.	.	.	.
face; Discoloration; red; left			.	.	.	.	.	.	.
vulva; Discoloration; brown			.	.	.	.	.	.	.
STOMACH;			.	.	.	.	.	.	.
UTERUS;			+	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral			.	.	.	.	.	.	.
horn; Dilatation; bilateral			.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral			P	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE							
GROUP	5	5	5	5	5	5	5
REMOVAL REASON	S	S	S	S	S	S	S
ANIMAL	3	3	3	3	3	3	3
NUMBER	1	2	5	5	5	5	5
	9	0	1	2	3	4	5
ANIMAL IDENTIFICATION;	+	+	+	+	+	+	+
Submitted	P	P	P	P	P	P	P
BONE MARROW, FEMUR;	.	.	N	N	N	N	N
BONE MARROW SMEAR;	+	+	+	+	+	+	+
Submitted	P	P	P	P	P	P	P
ESOPHAGUS;	.	.	.	.	.	.	.
SMALL INTESTINE, DUODENUM;	N	N	.	.	.	.	.
SMALL INTESTINE, JEJUNUM;	N	N	.	.	.	.	.
LIVER;	.	.	.	.	.	.	.
medial lobe; Hernia	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia	.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;	.	.	.	.	.	.	.
MESENTERY;	.	.	.	.	.	.	.
fat; Mass; mottled	.	.	.	.	.	.	.
fat; Mass; red	.	.	.	.	.	.	.
fat; Mass; yellow	.	.	.	.	.	.	.
fat; Nodule; mottled	.	.	.	.	.	.	.
ORAL MUCOSA;	N	N	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE							
GROUP	5	5	5	5	5	5	5
REMOVAL REASON	S	S	S	S	S	S	S
ANIMAL	3	3	3	3	3	3	3
NUMBER	1	2	5	5	5	5	5
	9	0	1	2	3	4	5
SKIN;	.	+	.	.	.	.	.
Discoloration; red; bilateral	.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation	.	.	.	.	.	.	.
face; Alopecia	.	P	.	.	.	.	.
face; Discoloration; red; bilateral	.	.	.	.	.	.	.
face; Discoloration; red; left	.	.	.	.	.	.	.
vulva; Discoloration; brown	.	.	.	.	.	.	.
STOMACH;	.	.	.	.	.	.	.
UTERUS;	.	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral	.	.	.	.	.	.	.
horn; Dilatation; bilateral	.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral	.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE								
GROUP	6	6	6	6	6	6	6	6
REMOVAL REASON	S	S	S	S	S	S	S	S
ANIMAL	3	3	3	3	3	3	3	3
NUMBER	8	8	8	8	9	9	9	9
	6	7	8	9	0	1	2	3
ANIMAL IDENTIFICATION;	+	+	+	+	+	+	+	+
Submitted	P	P	P	P	P	P	P	P
BONE MARROW, FEMUR;	.	.	.	.	.	.	.	.
BONE MARROW SMEAR;	.	.	.	.	.	+	+	+
Submitted	.	.	.	.	.	P	P	P
ESOPHAGUS;	N	N	N	N	N	N	N	N
SMALL INTESTINE, DUODENUM;	N	N	N	N	N	N	N	N
SMALL INTESTINE, JEJUNUM;	N	N	N	N	N	N	N	N
LIVER;	N	N	N	N	N	N	N	+
medial lobe; Hernia	.	.	.	.	.	.	.	.
median lobe; diaphragm; Hernia	.	.	.	.	.	.	.	P
LYMPH NODE, MESENTERIC;	N	N	N	N	N	N	N	N
MESENTERY;	.	.	+	.	.	.	.	.
fat; Mass; mottled	.	.	P	.	.	.	.	.
fat; Mass; red	.	.	.	.	.	.	.	.
fat; Mass; yellow	.	.	.	.	.	.	.	.
fat; Nodule; mottled	.	.	.	.	.	.	.	.
ORAL MUCOSA;	N	N	N	N	N	N	N	N

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE								
GROUP	6	6	6	6	6	6	6	6
REMOVAL REASON	S	S	S	S	S	S	S	S
ANIMAL	3	3	3	3	3	3	3	3
NUMBER	8	8	8	8	9	9	9	9
	6	7	8	9	0	1	2	3
SKIN;	.	+	+	.	.	.	.	+
Discoloration; red; bilateral	.	.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation	.	.	.	.	.	.	.	.
face; Alopecia	.	.	.	.	.	.	.	.
face; Discoloration; red; bilateral	.	P	.	.	.	.	.	.
face; Discoloration; red; left	.	.	P	.	.	.	.	.
vulva; Discoloration; brown	.	.	.	.	.	.	.	P
STOMACH;	N	N	N	N	N	N	N	N
UTERUS;	.	+	.	.	.	.	.	.
lumen; horn; Dilatation; bilateral	.	.	.	.	.	.	.	.
horn; Dilatation; bilateral	.	.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral	.	P	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE	GROUP	6	6	6	6	6	6	6
	REMOVAL REASON	S	S	S	S	S	S	S
	ANIMAL	3	3	4	4	4	4	4
	NUMBER	9	9	2	2	2	2	3
		4	5	6	7	8	9	0
ANIMAL IDENTIFICATION;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
BONE MARROW, FEMUR;		.	.	N	N	N	N	N
BONE MARROW SMEAR;		+	+	+	+	+	+	+
Submitted		P	P	P	P	P	P	P
ESOPHAGUS;		N	N	.	.	.	.	.
SMALL INTESTINE, DUODENUM;		N	N	.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	.	.	.	.	.
LIVER;		N	N	.	.	.	.	.
medial lobe; Hernia		.	.	.	.	.	.	.
median lobe; diaphragm; Hernia		.	.	.	.	.	.	.
LYMPH NODE, MESENTERIC;		N	N	.	.	.	.	.
MESENTERY;		.	.	.	.	.	.	.
fat; Mass; mottled		.	.	.	.	.	.	.
fat; Mass; red		.	.	.	.	.	.	.
fat; Mass; yellow		.	.	.	.	.	.	.
fat; Nodule; mottled		.	.	.	.	.	.	.
ORAL MUCOSA;		N	N	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Macroscopic Observations: Day 91

SEX: FEMALE							
GROUP	6	6	6	6	6	6	6
REMOVAL REASON	S	S	S	S	S	S	S
ANIMAL	3	3	4	4	4	4	4
NUMBER	9	9	2	2	2	2	3
	4	5	6	7	8	9	0
SKIN;	.	.	.	.	.	.	.
Discoloration; red; bilateral	.	.	.	.	.	.	.
No Macroscopic Correlation To Clinical Observation	.	.	.	.	.	.	.
face; Alopecia	.	.	.	.	.	.	.
face; Discoloration; red; bilateral	.	.	.	.	.	.	.
face; Discoloration; red; left	.	.	.	.	.	.	.
vulva; Discoloration; brown	.	.	.	.	.	.	.
STOMACH;	N	N	.	.	.	.	.
UTERUS;	.	+	.	.	.	.	.
lumen; horn; Dilatation; bilateral	.	P	.	.	.	.	.
horn; Dilatation; bilateral	.	.	.	.	.	.	.
horn; lumen; Dilatation; bilateral	.	.	.	.	.	.	.

+ = Tissue observation present N = No visible lesions P = Present – no grade or classification S = Scheduled euthanasia

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H2

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Macroscopic Observations: Day 8

	FEMALES					
Group:	1	2	3	4	5	6
Number of Animals:	5	5	5	5	5	5
ANIMAL IDENTIFICATION;						
Submitted	(5)	(5)	(5)	(5)	(5)	(5)
No Visible Lesions	0	0	0	0	0	0
Submitted	5	5	5	5	5	5
ESOPHAGUS;						
Submitted	(5)	(5)	(0)	(5)	(0)	(5)
No Visible Lesions	5	5	0	5	0	5
SMALL INTESTINE, DUODENUM;						
Submitted	(5)	(5)	(5)	(5)	(5)	(5)
No Visible Lesions	5	5	5	5	5	5
SMALL INTESTINE, JEJUNUM;						
Submitted	(5)	(5)	(5)	(5)	(5)	(5)
No Visible Lesions	5	5	5	5	5	5
LIVER;						
Submitted	(5)	(5)	(0)	(5)	(0)	(5)
No Visible Lesions	5	5	0	5	0	5
LYMPH NODE, MESENTERIC;						
Submitted	(5)	(5)	(0)	(5)	(0)	(5)
No Visible Lesions	5	5	0	5	0	5
ORAL MUCOSA;						
Submitted	(5)	(5)	(5)	(5)	(5)	(5)
No Visible Lesions	5	5	5	5	5	5
STOMACH;						
Submitted	(5)	(5)	(0)	(5)	(0)	(5)
No Visible Lesions	5	5	0	5	0	5

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H2

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Macroscopic Observations: Day 91

	FEMALES					
Group:	1	2	3	4	5	6
Number of Animals:	15	15	15	15	15	15
ANIMAL IDENTIFICATION;						
Submitted	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	0	0	0	0	0	0
Submitted	15	15	15	15	15	15
BONE MARROW, FEMUR;						
Submitted	(5)	(5)	(5)	(5)	(5)	(5)
No Visible Lesions	5	5	5	5	5	5
BONE MARROW SMEAR;						
Submitted	(10)	(10)	(10)	(10)	(10)	(10)
No Visible Lesions	0	0	0	0	0	0
Submitted	10	10	10	10	10	10
ESOPHAGUS;						
Submitted	(10)	(10)	(0)	(10)	(0)	(10)
No Visible Lesions	10	10	0	10	0	10
SMALL INTESTINE, DUODENUM;						
Submitted	(10)	(10)	(10)	(10)	(10)	(10)
No Visible Lesions	10	10	10	10	10	10
SMALL INTESTINE, JEJUNUM;						
Submitted	(10)	(10)	(10)	(10)	(10)	(10)
No Visible Lesions	10	10	10	10	10	10
LIVER;						
Submitted	(10)	(10)	(0)	(10)	(0)	(10)
No Visible Lesions	10	10	0	9	0	9
Hernia; medial lobe	0	0	0	1	0	0
Hernia; median lobe; diaphragm	0	0	0	0	0	1

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H2

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Macroscopic Observations: Day 91

	FEMALES					
Group:	1	2	3	4	5	6
Number of Animals:	15	15	15	15	15	15
LYMPH NODE, MESENTERIC;						
Submitted	(10)	(10)	(0)	(10)	(0)	(10)
No Visible Lesions	10	10	0	10	0	10
MESENTERY;						
Submitted	(0)	(0)	(2)	(0)	(3)	(1)
No Visible Lesions	0	0	0	0	0	0
Mass; mottled; fat	0	0	0	0	0	1
Mass; red; fat	0	0	0	0	1	0
Mass; yellow; fat	0	0	2	0	1	0
Nodule; mottled; fat	0	0	0	0	1	0
ORAL MUCOSA;						
Submitted	(10)	(10)	(10)	(10)	(10)	(10)
No Visible Lesions	10	10	10	10	10	10
SKIN;						
Submitted	(0)	(0)	(1)	(0)	(8)	(3)
No Visible Lesions	0	0	0	0	0	0
Alopecia; face	0	0	0	0	6	0
Discoloration; brown; vulva	0	0	0	0	0	1
Discoloration; red; bilateral	0	0	1	0	0	0
Discoloration; red; face; bilateral	0	0	0	0	0	1
Discoloration; red; face; left	0	0	0	0	0	1
No Macroscopic Correlation To Clinical Observation	0	0	0	0	2	0
STOMACH;						
Submitted	(10)	(10)	(0)	(10)	(0)	(10)
No Visible Lesions	10	10	0	10	0	10
UTERUS;						
Submitted	(0)	(1)	(0)	(0)	(1)	(2)
No Visible Lesions	0	0	0	0	0	0
Dilatation; bilateral; lumen; horn	0	0	0	0	0	1
Dilatation; bilateral; horn	0	1	0	0	0	0
Dilatation; bilateral; horn; lumen	0	0	0	0	1	1

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 8

SEX: FEMALE						
	GROUP	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	.	.	.	.	.
	NUMBER	7	7	7	7	7
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 8

SEX: FEMALE	GROUP	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	1	1	1	1	1
	NUMBER	4	4	4	4	5
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 8

SEX: FEMALE	GROUP	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	2	2	2	2	2
	NUMBER	2	2	2	2	2
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 8

SEX: FEMALE	GROUP	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	2	2	2	2	3
	NUMBER	9	9	9	9	0
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		N	+	N	N	+
Apoptosis		.	1	.	.	.
crypt; Hyperplasia		.	.	.	.	1
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 8

SEX: FEMALE						
	GROUP	5	5	5	5	5
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	3	3	3	3	3
	NUMBER	7	7	7	7	7
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		1	2	1	1	.
crypt; Hyperplasia		3	2	3	2	2
lamina propria; Infiltration Cellular; histiocytic		.	2	1	.	1
villus; Atrophy		2	2	1	1	1
SMALL INTESTINE, JEJUNUM;		+	+	+	+	+
Apoptosis		.	1	1	.	1
crypt; Hyperplasia		1	2	2	1	1
villus; Atrophy		1	1	1	1	.
ORAL MUCOSA;		N	N	N	N	N

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 8

SEX: FEMALE						
	GROUP	6	6	6	6	6
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	4	4	4	4	4
	NUMBER	4	4	4	4	5
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		1	.	1	1	.
crypt; Hyperplasia		2	1	2	2	2
lamina propria; Infiltration Cellular; histiocytic		1	1	1	1	.
villus; Atrophy		.	.	1	1	.
SMALL INTESTINE, JEJUNUM;		+	+	+	+	+
Apoptosis		.	.	1	1	.
crypt; Hyperplasia		1	2	2	2	1
villus; Atrophy		.	1	.	1	1
ORAL MUCOSA;		N	N	N	N	N

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE	GROUP	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	.	.	.	.	.
	NUMBER	1	1	1	1	1
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE	GROUP	1	1	1	1	1
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	.	.	.	.	.
	NUMBER	1	1	1	1	2
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification N = No visible lesions 1= Minimal 3= Moderate
+ = Tissue observation present S = Scheduled euthanasia 2= Mild 4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE	GROUP	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	.	.	.	.	.
	NUMBER	8	8	8	8	9
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	+
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	1
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	2	2	2	2	2
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	.	.	.	.	.
	NUMBER	9	9	9	9	9
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	+	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	P	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	1	1	1	1	1
	NUMBER	6	6	6	6	6
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		+	+	+	N	N
Inflammation; chronic		.	.	2	.	.
Apoptosis		.	.	1	.	.
crypt; Hyperplasia		1	1	.	.	.
crypt; Depletion		.	.	3	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
submucosal; Fibrosis		.	.	3	.	.
peyers patch; Mineralization		.	1	.	.	.
villus; Atrophy		.	.	3	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	+	.	.	.
Necrosis, Fat		.	4	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	N	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	3	3	3	3	3
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	1	1	1	1	1
	NUMBER	6	6	6	6	7
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		N	N	N	N	N
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		N	N	N	N	N
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	+	.	.	.
Necrosis, Fat		.	4	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	2	2	2	2	2
	NUMBER	3	3	3	3	4
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		1	.	.	1	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		2	2	2	1	2
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		+	N	N	+	N
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		.	.	.	1	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		1	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	4	4	4	4	4
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	2	2	2	2	2
	NUMBER	4	4	4	4	4
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		+	+	N	+	+
Apoptosis		1	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		2	2	.	2	1
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		+	N	+	N	N
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		1	.	1	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	+	.
Hepatodiaphragmatic Nodule		.	.	.	P	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	.	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	5	5	5	5	5
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	3	3	3	3	3
	NUMBER	1	1	1	1	1
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		1	1	.	.	2
crypt; Hyperplasia		.	1	1	1	.
lamina propria; Infiltration Cellular; histiocytic		3	4	3	3	2
villus; Atrophy		.	2	.	.	.
SMALL INTESTINE, JEJUNUM;		+	+	+	+	+
Inflammation; chronic		.	.	.	.	.
Apoptosis		1	.	.	.	.
crypt; Hyperplasia		.	1	.	1	1
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		1	.	2	.	.
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		+	.	.	.	.
Necrosis, Fat		2	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	+	+	+	.
Inflammation; chronic		.	.	.	1	.
hair follicle; Atrophy		.	3	3	2	.
UTERUS;		.	+	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	P	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	5	5	5	5	5
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	3	3	3	3	3
	NUMBER	1	1	1	1	2
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		.	1	.	.	.
crypt; Hyperplasia		1	.	.	.	1
lamina propria; Infiltration Cellular; histiocytic		3	4	4	3	3
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		+	+	+	+	+
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	1
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		1	1	1	1	1
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		+	.	+	.	.
Necrosis, Fat		2	.	4	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		+	.	+	.	+
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		2	.	3	.	1
UTERUS;		.	.	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	6	6	6	6	6
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	3	3	3	3	3
	NUMBER	8	8	8	8	9
		6	7	8	9	0
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		2	.	1	.	1
crypt; Hyperplasia		1	1	.	1	.
lamina propria; Infiltration Cellular; histiocytic		4	3	4	3	2
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		+	+	+	+	+
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	1	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		1	1	2	1	1
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	.	.	.
Hepatodiaphragmatic Nodule		.	.	.	.	.
MESENTERY;		.	.	+	.	.
Necrosis, Fat		.	.	4	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	N	N	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	+	.	.	.
No Microscopic Correlation To Macroscopic Observation		.	P	.	.	.

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H3

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Microscopic Observations: Day 91

SEX: FEMALE						
	GROUP	6	6	6	6	6
	REMOVAL REASON	S	S	S	S	S
	ANIMAL	3	3	3	3	3
	NUMBER	9	9	9	9	9
		1	2	3	4	5
SMALL INTESTINE, DUODENUM;		+	+	+	+	+
Apoptosis		1	1	1	1	.
crypt; Hyperplasia		.	.	.	1	.
lamina propria; Infiltration Cellular; histiocytic		4	4	4	1	4
villus; Atrophy		.	.	.	.	.
SMALL INTESTINE, JEJUNUM;		+	+	+	N	+
Inflammation; chronic		.	.	.	.	.
Apoptosis		.	.	.	.	.
crypt; Hyperplasia		.	.	.	.	.
crypt; Depletion		.	.	.	.	.
lamina propria; Infiltration Cellular; histiocytic		2	1	2	.	2
submucosal; Fibrosis		.	.	.	.	.
peyers patch; Mineralization		.	.	.	.	.
villus; Atrophy		.	.	.	.	.
LIVER;		.	.	+	.	.
Hepatodiaphragmatic Nodule		.	.	P	.	.
MESENTERY;		.	.	.	.	.
Necrosis, Fat		.	.	.	.	.
ORAL MUCOSA;		N	N	N	N	N
SKIN;		.	.	N	.	.
Inflammation; chronic		.	.	.	.	.
hair follicle; Atrophy		.	.	.	.	.
UTERUS;		.	.	.	.	+
No Microscopic Correlation To Macroscopic Observation		.	.	.	.	P

P= Present - no grade or classification
+ = Tissue observation present
N = No visible lesions
S = Scheduled euthanasia
1= Minimal
2= Mild
3= Moderate
4= Marked

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Microscopic Observations: Day 8

	FEMALES					
Group:	1	2	3	4	5	6
Number of Animals:	5	5	5	5	5	5
SMALL INTESTINE, DUODENUM;						
Examined	(5)	(5)	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	3	0	0
Hyperplasia; crypt	0	0	0	1	5	5
Infiltration Cellular; histiocytic; lamina propria	0	0	0	0	3	4
Atrophy; villus	0	0	0	0	5	2
Apoptosis	0	0	0	1	4	3
SMALL INTESTINE, JEJUNUM;						
Examined	(5)	(5)	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5	0	0
Hyperplasia; crypt	0	0	0	0	5	5
Atrophy; villus	0	0	0	0	4	3
Apoptosis	0	0	0	0	3	2
ORAL MUCOSA;						
Examined	(5)	(5)	(5)	(5)	(5)	(5)
Within Normal Limits	5	5	5	5	5	5

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Microscopic Observations: Day 91

	FEMALES					
Group:	1	2	3	4	5	6
Number of Animals:	10	10	10	10	10	10
SMALL INTESTINE, DUODENUM;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	10	10	10	1	0	0
Hyperplasia; crypt	0	0	0	0	5	4
Infiltration Cellular; histiocytic; lamina propria	0	0	0	9	10	10
Atrophy; villus	0	0	0	0	1	0
Apoptosis	0	0	0	3	4	7
SMALL INTESTINE, JEJUNUM;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	10	9	7	6	0	1
Fibrosis; submucosal	0	0	1	0	0	0
Hyperplasia; crypt	0	0	2	0	3	1
Infiltration Cellular; histiocytic; lamina propria	0	0	0	3	7	9
Inflammation; chronic	0	0	1	0	0	0
Mineralization; peyers patch	0	1	1	1	0	0
Atrophy; villus	0	0	1	0	0	0
Depletion; crypt	0	0	1	0	0	0
Apoptosis	0	0	1	0	2	0
LIVER;						
Examined	(0)	(0)	(0)	(1)	(0)	(1)
Within Normal Limits	0	0	0	0	0	0
Hepatodiaphragmatic Nodule	0	0	0	1	0	1
MESENTERY;						
Examined	(0)	(0)	(2)	(0)	(3)	(1)
Within Normal Limits	0	0	0	0	0	0
Necrosis, Fat	0	0	2	0	3	1

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table H4

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Microscopic Observations: Day 91

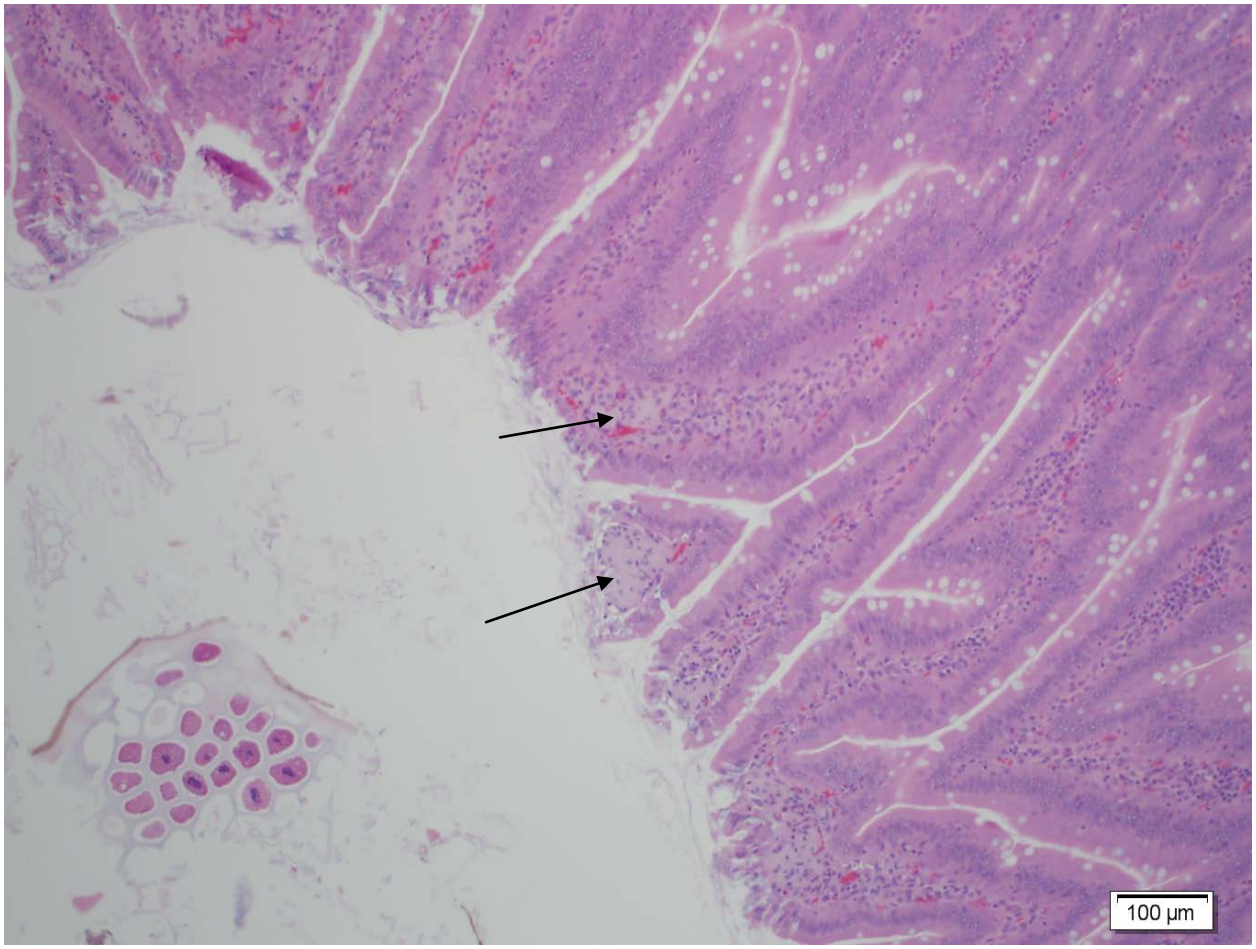
	FEMALES					
Group:	1	2	3	4	5	6
Number of Animals:	10	10	10	10	10	10
ORAL MUCOSA;						
Examined	(10)	(10)	(10)	(10)	(10)	(10)
Within Normal Limits	10	10	10	10	10	10
SKIN;						
Examined	(0)	(0)	(1)	(0)	(6)	(3)
Within Normal Limits	0	0	1	0	0	3
Inflammation; chronic	0	0	0	0	1	0
Atrophy; hair follicle	0	0	0	0	6	0
UTERUS;						
Examined	(0)	(1)	(0)	(0)	(1)	(2)
Within Normal Limits	0	0	0	0	0	0
No Microscopic Correlation To Macroscopic Observation	0	1	0	0	1	2

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H1

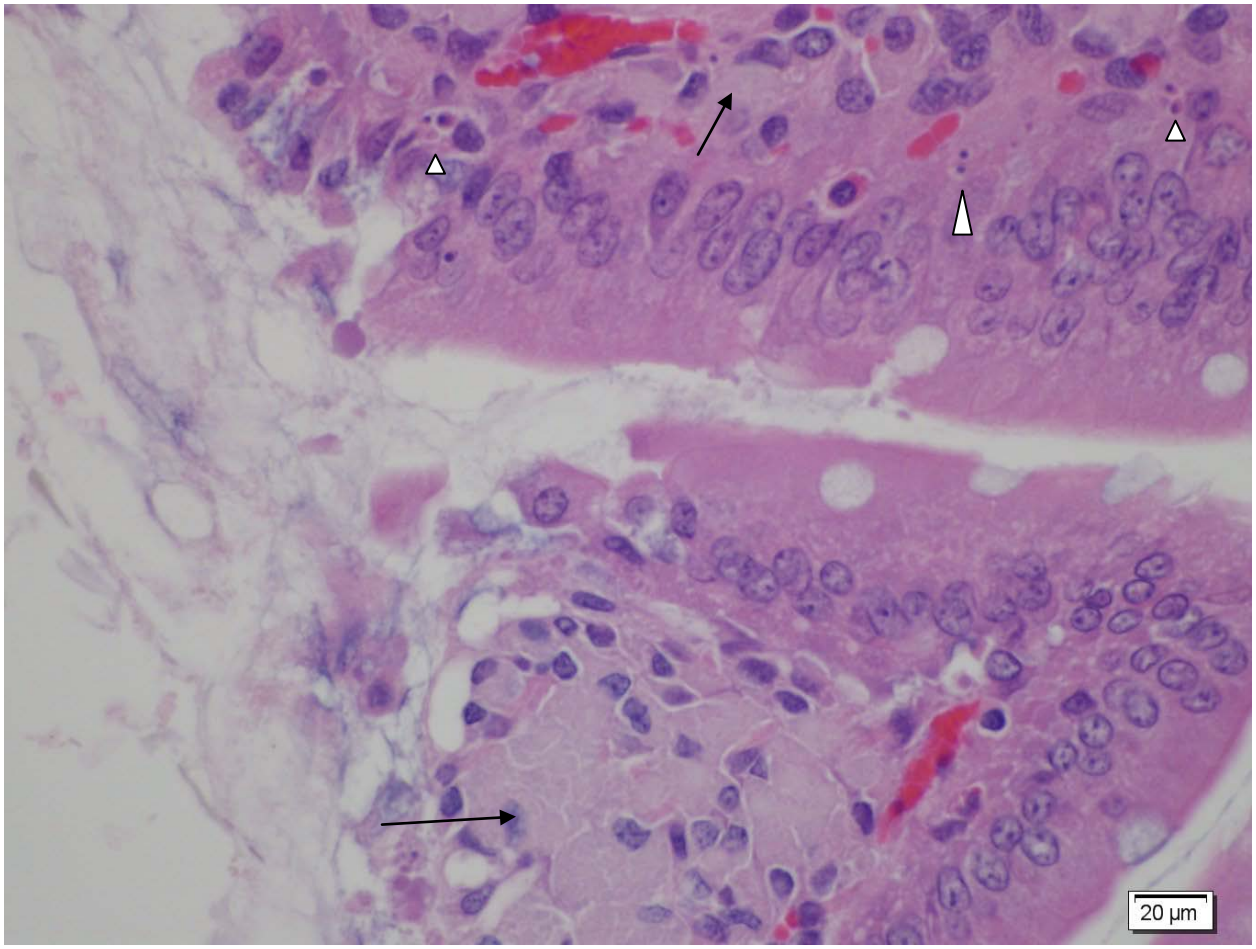


Animal 6F386 (520 mg/L SDD) Day 91: Duodenum. Note the marked histiocytic cell infiltration in the lamina propria of the villous tips (arrows).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H2

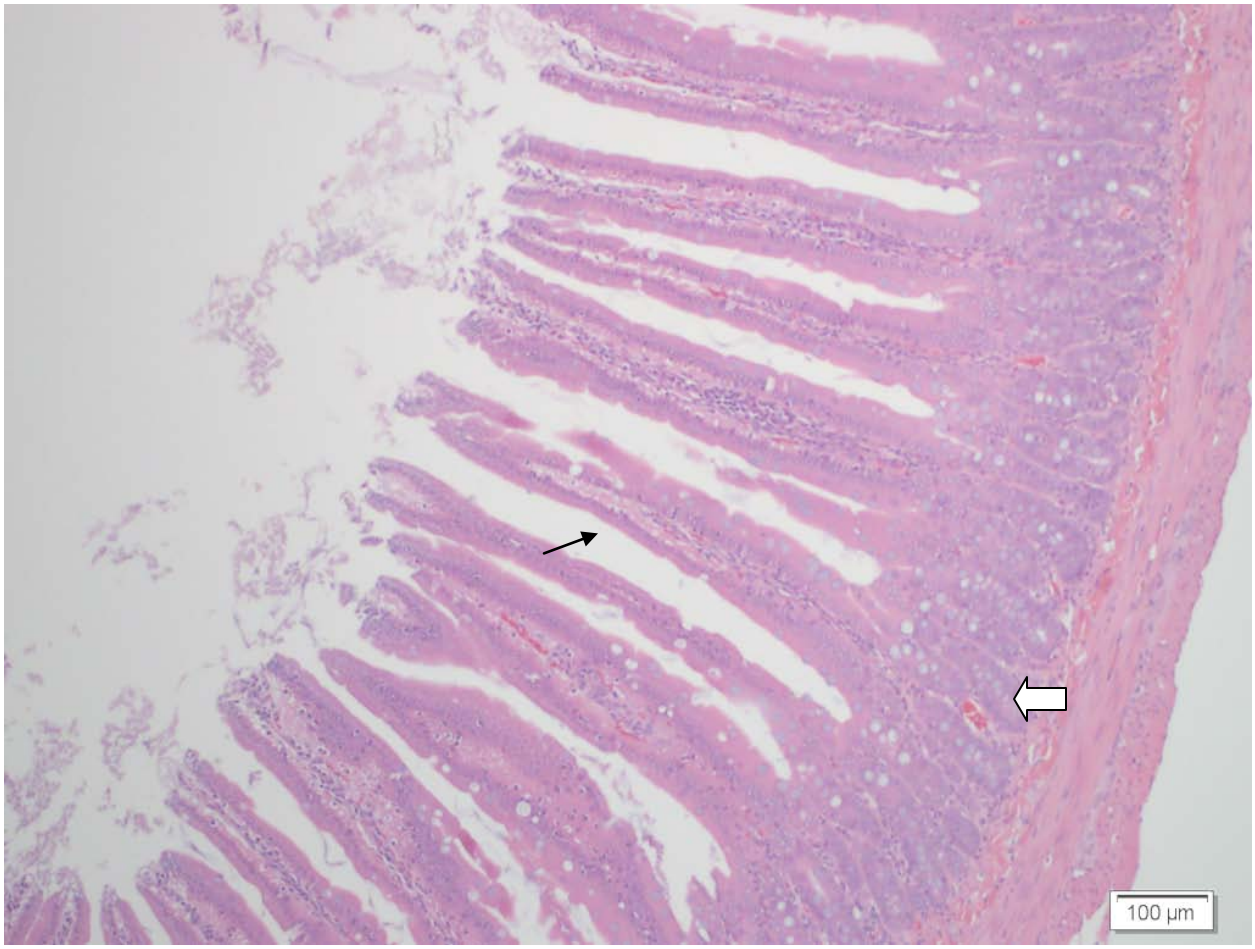


Animal 6F386 (520 mg/L SDD) Day 91: Duodenum. Note the marked histiocytic cell infiltration in the lamina propria of the villous tips (arrow) and the apoptotic bodies (arrow heads).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H3

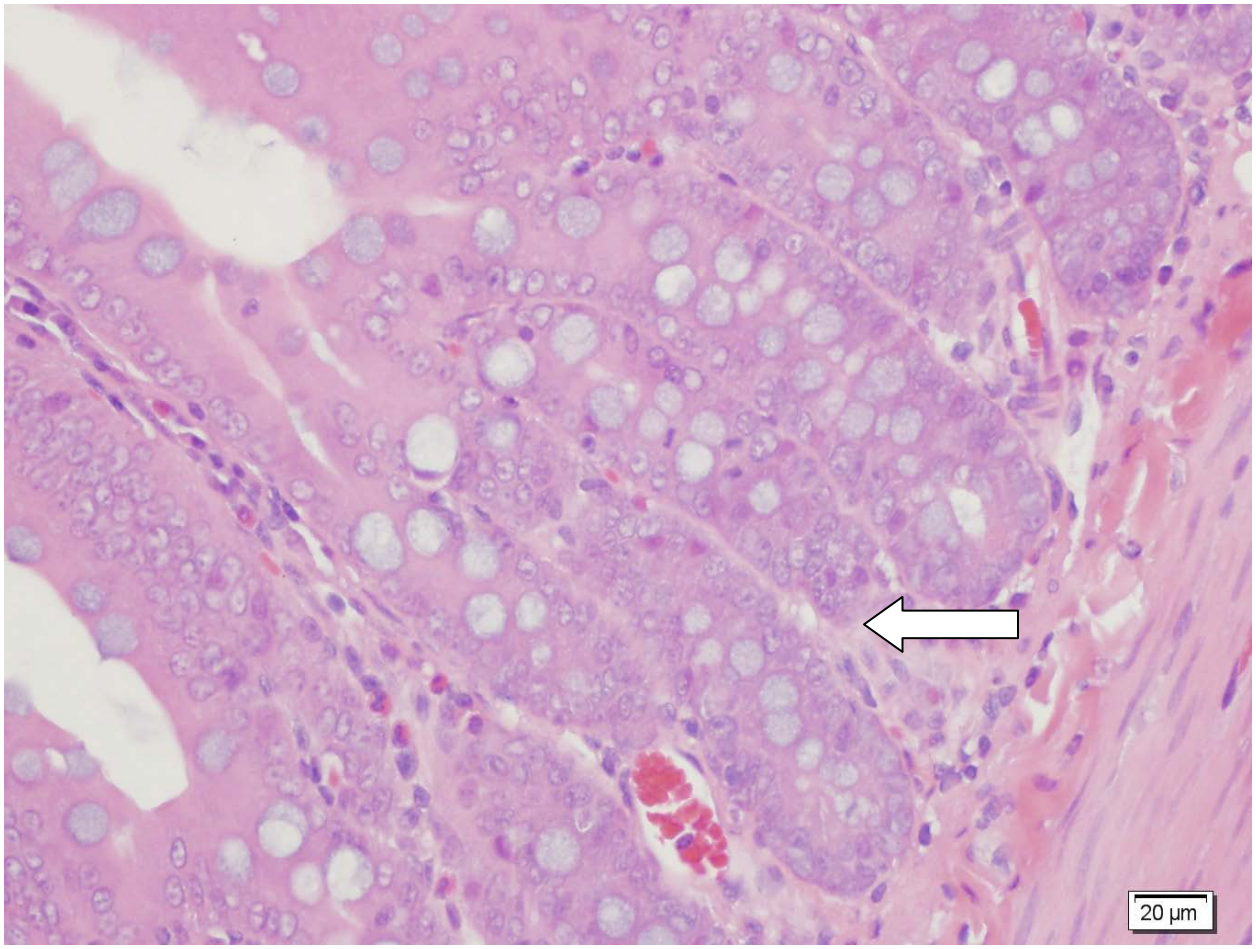


Animal 1F13 (0 mg/L SDD) Day 91: Duodenum. Normal duodenum. Note the crypt epithelium (large arrow) and long thin villi (small arrow).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H4

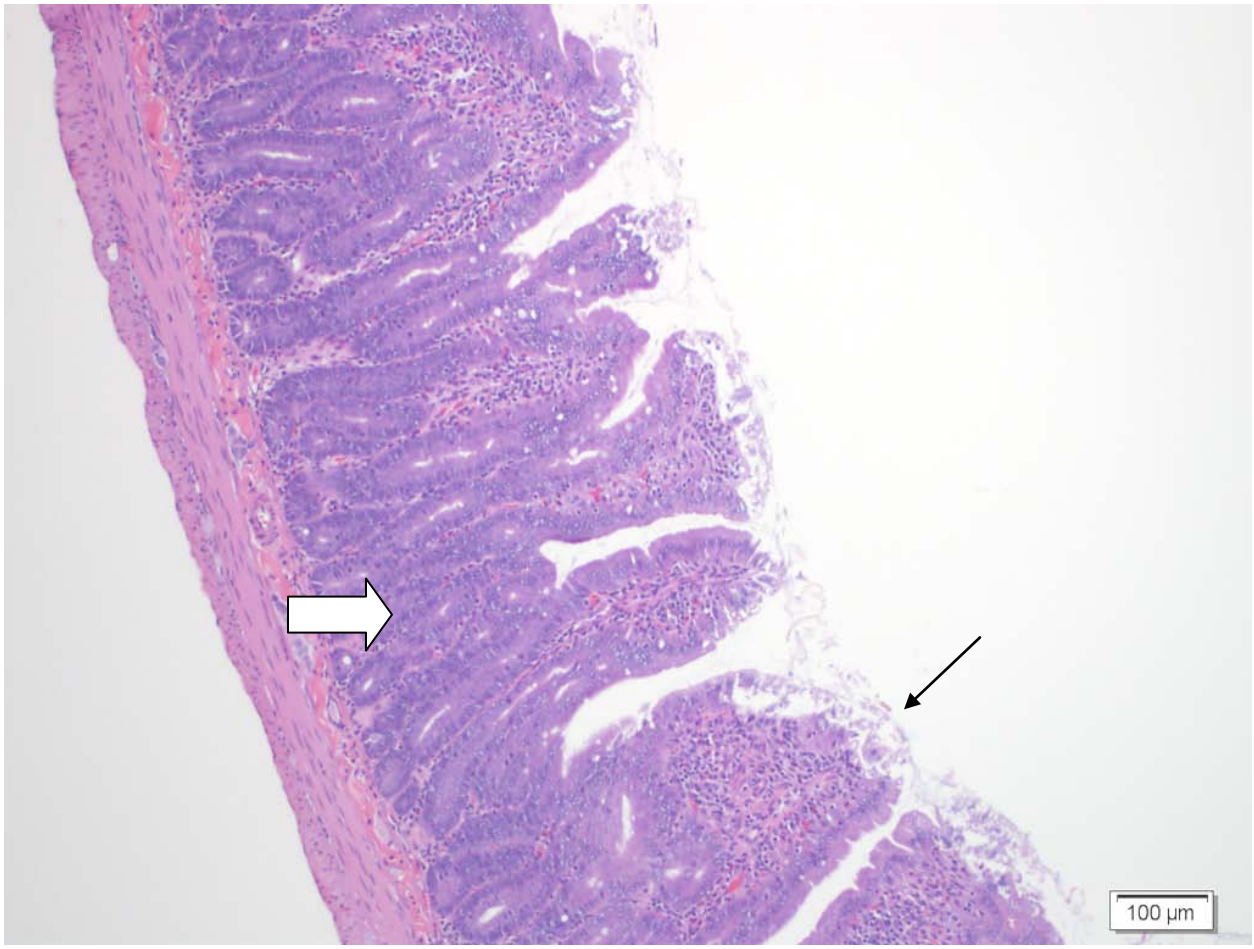


Animal 1F13 (0 mg/L SDD) Day 91: Duodenum. Normal duodenum. Note the crypt epithelium (arrow).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H5

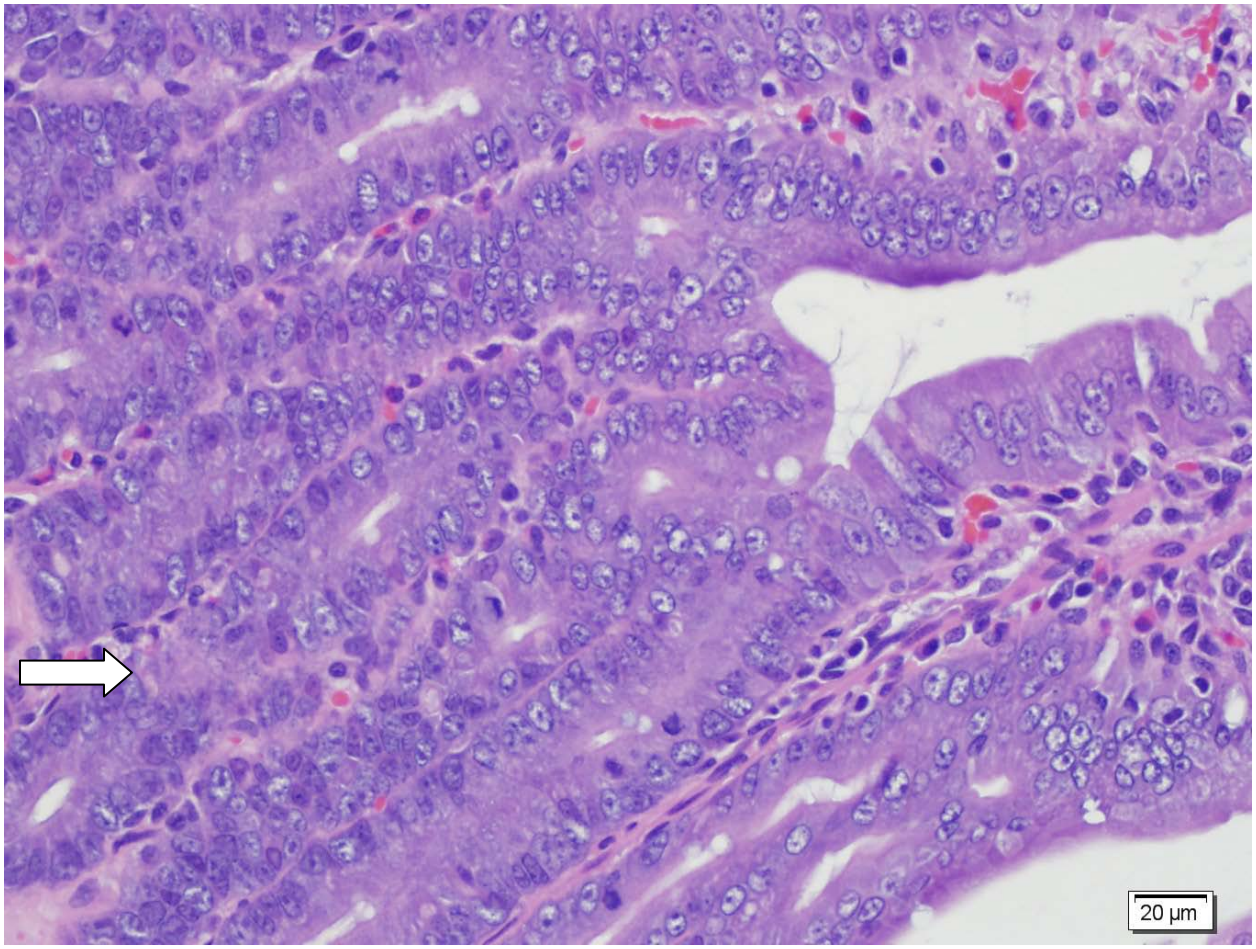


Animal 5F371 (170 mg/L SDD) Day 8: Duodenum. Note the hyperplastic crypt epithelium with increased basophilia (large arrow) and the short, blunt, atrophied villi (small arrow).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H6

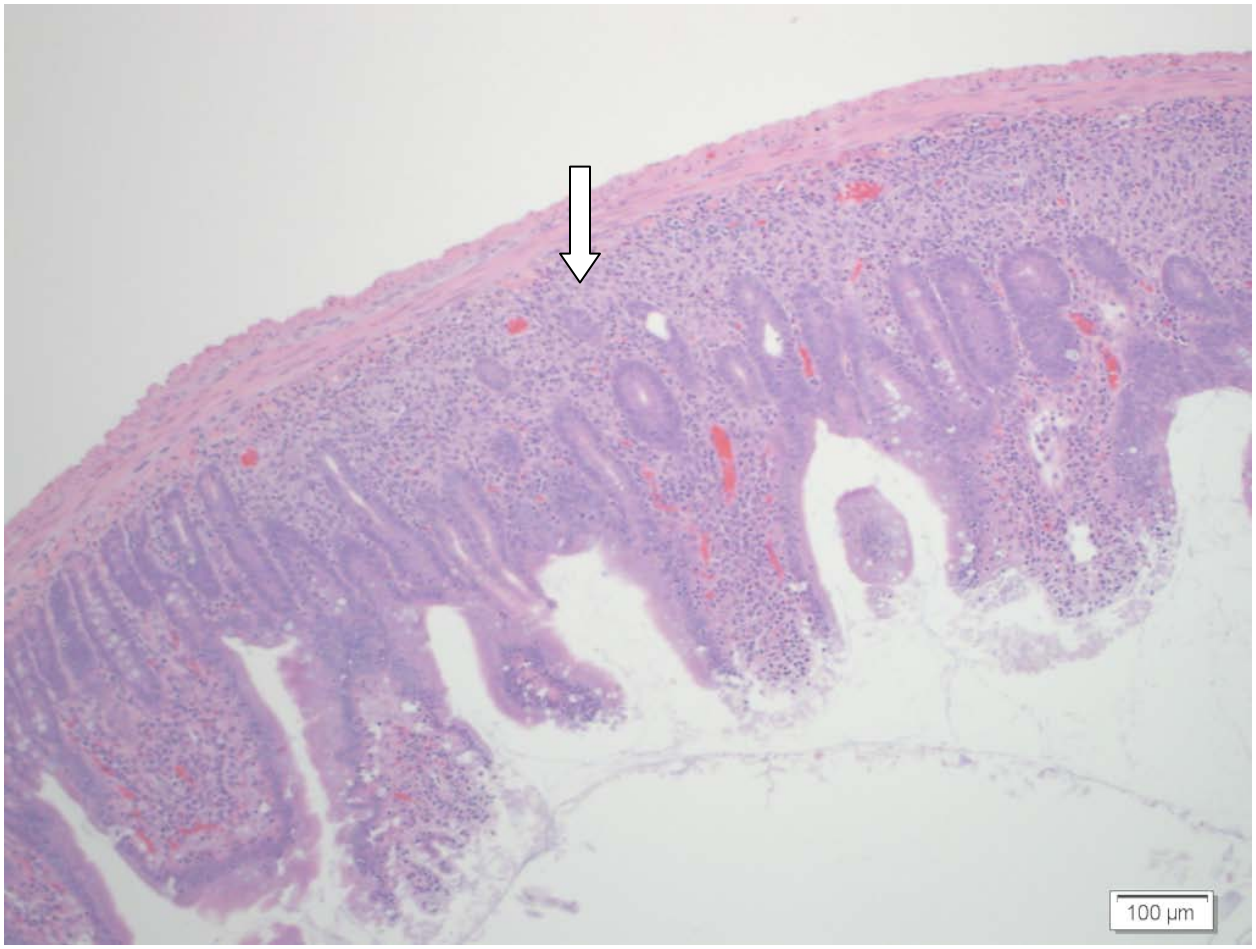


Animal 5F71 (170 mg/L SDD) Day 8: Duodenum. Note the hyperplastic crypt epithelium with increased basophilia (arrow).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H7

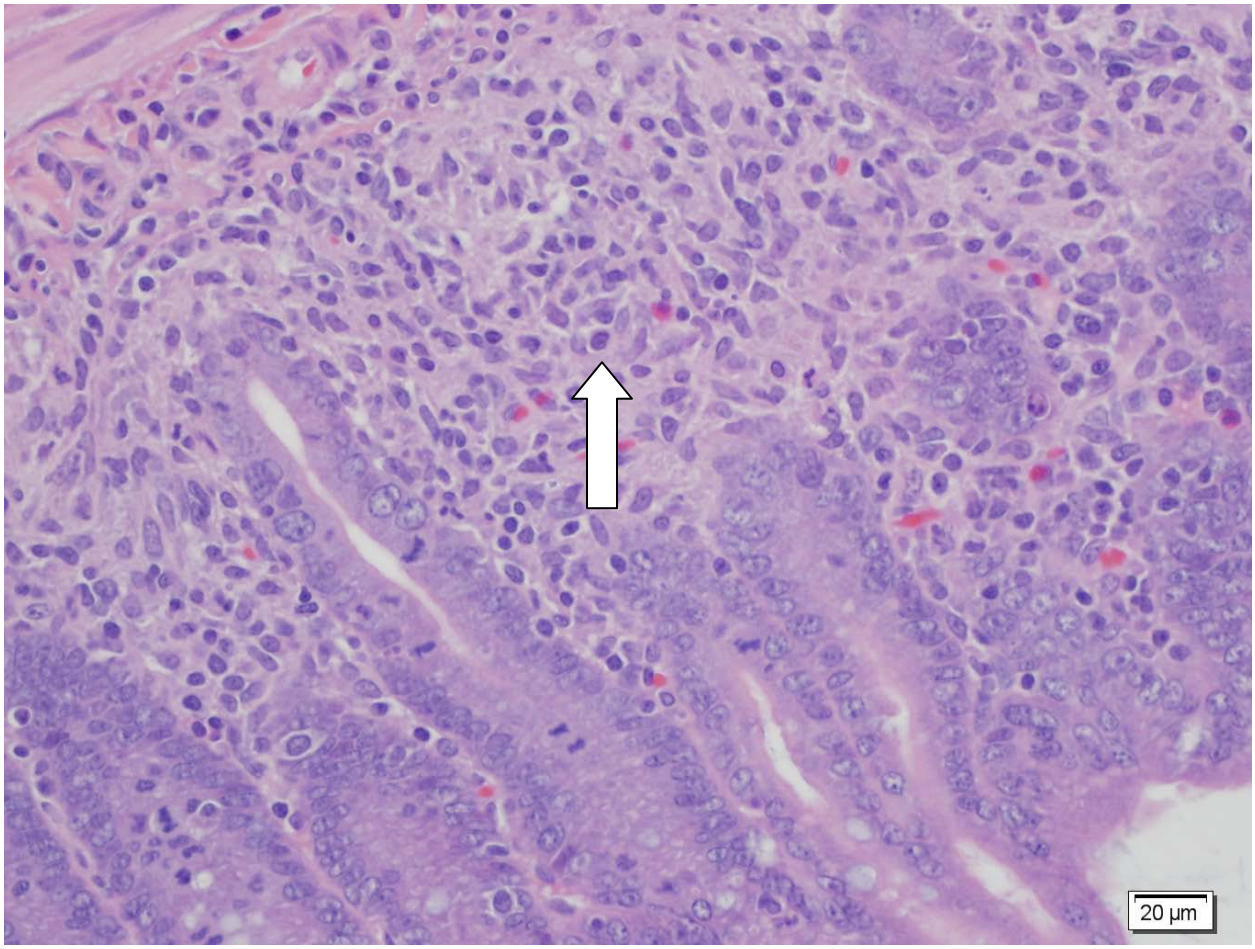


Animal 3F163 (4 mg/L SDD) Day 91: Jejunum. Note the crypt depletion and submucosal fibrosis (arrow).

90-Day Repeat Dose Toxicity of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Photomicrographs of Selected Tissues with Lesions

Figure H8



Animal 3F163 (4 mg/L SDD) Day 91: Jejunum. The depleted crypt epithelium was replaced by submucosal fibrosis and chronic inflammation (arrow).

Appendix I

Clinical Pathology Contributing Scientist Report

Clinical Pathology Contributing Scientist Report for

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate Administered in Drinking Water to Fischer Rats

Submitted by:

Brenda Yamamoto, D.V.M., Ph.D., D.A.C.V.P.
Clinical Pathologist
Southern Research Institute
Birmingham, Alabama

Southern Research Study Number: 13026.01.02

June 15, 2011

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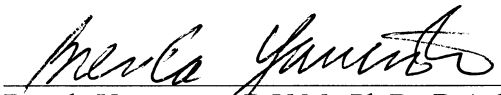

6-15-11

Brenda Yamamoto, D.V.M., Ph.D., D.A.C.V.P.
Clinical Pathologist
Southern Research Institute

Date

2.0 SUMMARY

The objective of this study was to evaluate the toxicity and potential mechanisms of action of sodium dichromate dihydrate (SDD) administered in drinking water to rats for 90 days. In female Fischer rats administered sodium dichromate dihydrate (SDD) in the drinking water for 91 days, decreases in mean serum iron levels relative to the control group mean were seen at dosages $\geq$ 60 mg/L on Day 91. The decrease, however, was statistically significant only at dosages of 170 or 520 mg/L SDD. Results from evaluation of Prussian-blue stained bone marrow smears from Day 91 were supportive for decreased iron content/storage with 170 and 520 mg/L SDD administration. Taken together, the findings suggest that SDD administration at dosages $\geq$ 170 mg/L for 90 days to female Fischer rats is associated with disturbances of iron metabolism..

3.0 METHODS AND MATERIALS

Female Fischer rats were assigned to six dosage groups receiving 0 mg/L (water only; control Group 1) or 0.3, 4, 60, 170, 520 mg/L of SDD (Groups 2-6, respectively) in their drinking water for 91 days. Serum iron levels were determined from five rats per group on Day 91. Additionally, on the same rats, bone marrow smears were collected, stained with Prussian blue stain, and used to semi-quantitatively assess iron content based upon a histological grading scheme (Table CP1).[(1)] Note: serum iron levels were determined in one set of rats from the Toxicology and Histopathology subgroup (11/2/2010), but the bone marrow smears could not be evaluated due to low cellularity, a complication attributed to the staining process. Thus, serum iron and bone marrow iron content were assessed from a second set of rats from the Mutation Analysis subgroup (11/22/2010).

Table CP1. Semi-quantitative grading scheme for iron in a bone marrow smear based upon Gale et.al.[(1)]

Grade	Iron content	Qualification of assessment
0	Slight	No iron visible under high power magnification (1000×)
1	Very slight	Small iron particles barely visible under high power magnification (1000×)
2	Slight	Small iron particles with sparse distribution (100×)
3	Moderate	Many small iron particles present in reticulum cells throughout marrow fragments (100×)
4	Moderately heavy	Large iron particles with tendency to aggregate in clumps present throughout the marrow fragments (100×)
5	Heavy	Large, dense clumps of iron present throughout the marrow fragments (100×)
6	Very heavy	Very large intra- and extra-cellular iron deposits present throughout the marrow fragments obscuring cellular detail (100×)

Fe = iron

Individual serum iron values and the mean for each group were recorded and/or calculated with the Provantis 7 (Instem; Staffordshire, UK) software. The two serum iron data sets were combined for statistical analysis, and the mean value for Group 1 was compared to Groups 2-6 by a one-way Analysis of Variance (ANOVA) followed by the Dunnett's test. EXCEL 2007 (Microsoft, Inc., Redmond, WA) was used to calculate the percent change (%Δ) in individual and mean serum iron levels for the SDD administration groups relative to the control group mean using the following formula: %Δ = [('X' – control mean)/control mean] × 100. 'X' represents either the individual or mean serum iron level. Qualifiers used to describe increases in serum iron include: minimal (30-45%), mild (46-60%), moderate (61-75%), and marked (≥76%). Qualifiers used to describe decreases in serum iron include: minimal (25-30%), mild (31-35%), moderate (36-40%), and marked (≥41%). Additionally, a 2.5-97.5% reference range was determined using the serum iron values determined from the ten control rats (Group 1) analyzed on 11/2/2010 and 11/22/2010. The numbers of individuals from Groups 2–6 outside of this range were determined. The analysis of the bone marrow iron content was done by comparing the SDD administration Groups 2-6 with that of the control Group 1 rats. Note: individual animals are identified by group number, sex, and animal identification number (e.g., 2F126 = Group 2, Female, animal identification number 126).

4.0 RESULTS

Serum Iron

The individual serum iron levels from both data sets (11/2/2010 and 11/22/2010) are presented in Table I1; group mean serum iron levels from both data sets (11/2/2010 and 11/22/2010) are presented in Table I2. Table CP2 presents the percent change in individual serum iron levels and Table CP3 presents the percent change in group mean serum iron levels relative to the control group mean. Overall, the mean serum iron levels were decreased relative to the control group mean with 60 mg/L or greater SDD administration. The decrease, however, plateaus and does not show a dose-dependent relationship. Statistical significance ($p<0.05$) was present in the mean serum iron levels only with 170 mg/L or greater SDD administration.

Serum iron levels were determined for two sets of five rats per group measured on 11/2/2010 (set 1) and 11/22/2010 (set 2). The mean serum iron level and data were assessed separately for each set of rats. In the first set, 2/5 rats (4F242 and 4F244) with 60 mg/L SDD administration had marked decreases in serum iron relative to the control group mean. One of five rats (5F316) had a moderate serum iron decrease and 1/5 rats (5F317) had a marked serum iron decrease with 170 mg/L SDD administration. A moderate decrease was present in 1/5 rats (6F392) with 520 mg/L SDD administration. Decreases of -14, -24, and -20% relative to the control mean were observed with 60, 170, and 520 mg/L SDD administration, respectively. In the second set of rats, serum iron levels were minimally increased in 3/5 rats (2F126, 2F128, and 2F130) and mildly increased in 1/5 rats (2F129) with 0.3 mg/L SDD administration relative to the control group mean. The absence of similar elevations in the first set of rats analyzed renders the apparent increases to be of questionable or limited significance. A moderate decrease in serum iron was seen for 1/5 rats (4F277), and minimal decrease in serum iron was seen for 2/5 rats (5F352 and 5F354) and 2/5 rats (6F427 and 6F430) with 60, 170, and 520 mg/L SDD administration, respectively. In the second set of rats, decreases of -17, -15, and -21% relative to the control mean was observed with 60, 170, and 520 mg/L SDD administration, respectively.

Additionally, the data from the two set of rats were grouped and the serum iron levels for the individual rats in the SDD administration groups (2–6) were compared to a 2.5–97.5 % reference interval established from the ten control group individuals, 229–376 µg/dL. Based upon this

reference interval, 4/10 rats in the 0.3 mg/L SDD administration group had serum iron levels that were increased. Decreases were present in 0/10, 0/10, 3/10, 2/10, and 3/10 rats with 0.3, 4, 60, 170, and 520 mg/L SDD administration, respectively. Statistical analysis comparing the mean serum iron level from Group 1 to the mean serum iron levels of Groups 2-6, indicated there was a significant decrease ($p<0.05$) with 170 and 520 mg/L SDD administration.

Table CP2. Percent change in the individual serum iron levels relative to the Group 1 (control) mean

Group	SDD Concentration (mg/L)	11/2/2010		11/22/2010	
		Animal ID	Serum Fe (%Δ)	Animal ID	Serum Fe (%Δ)
1	0	16	16	51	-30
		17	-5	52	10
		18	-4	53	-10
		19	-8	54	22
		20	1	55	8
2	0.3	91	-10	126	40
		92	11	127	15
		93	4	128	33
		94	-16	129	54
		95	12	130	36
3	4	166	-12	201	13
		167	-9	202	-7
		168	-3	203	-10
		169	-5	204	9
		170	0	205	14
4	60	241	10	276	6
		242	-43	277	-36
		243	15	278	-15
		244	-42	279	-19
		245	-10	280	-21
5	170	316	-37	351	-1
		317	-41	352	-26
		318	-27	353	-6
		319	-12	354	-26
		320	-4	355	-18
6	520	391	-10	426	-11
		392	-37	427	-28
		393	-23	428	-24
		394	-23	429	-14
		395	-4	430	-29

Abbreviations: Fe = iron; %Δ = percent change; ID = identification

Table CP3. Percent change in the mean serum iron levels relative to the Group 1 (control) mean

Group	SDD Concentration (mg/L)	Serum Fe (%Δ)	
		11/2/2010	11/22/2010
1	0	NA	NA
2	0.3	0	36
3	4	-6	4
4	60	-14	-17
5	170	-24	-15
6	520	-20	-21

Abbreviations: Fe = iron; %Δ = percent change; NA = not applicable

Bone Marrow Iron Staining

The results of the Prussian blue-stained bone marrow smears from the second set of Day 91 rats are presented in Table CP4. Rats with 170 and 520 mg/L SDD administration appear to have lower quantities of iron present on the bone marrow smear.

Based upon the grading scheme of Gale et.al.,[(1)] 1/5 and 4/5 control group rats had a Grade 2-3 and Grade 3 levels of detectable iron on the bone marrow smear, respectively. Rats in the 0.3, 4, and 60 mg/L SDD administration groups had similar results to the control group rats. In contrast, 3/5 rats in the 170 mg/L SDD administration group had Grade 2-3 levels of detectable iron on the bone marrow smear. Five of five rats in the 520 mg/L SDD administration group had Grade 2 levels of detectable iron on the bone marrow smear.

Table CP4. Estimate of iron content from Day 91 bone marrow smears

Animal ID	Quality of smear (cellularity)	Fe content in smear/fragment(s) (Grade 0 - 6)	Fe present within macrophage(s) (- or +)
1F51	low but adequate	2 - 3	+
1F52	adequate	3	+
1F53	adequate	3	+
1F54	adequate	3	+
1F55	adequate	3	+
2F126	low but adequate	3	+
2F127	adequate	3	+
2F128	adequate	3	+
2F129	adequate	3	+
2F130	adequate	3	+
3F201	adequate	3	+
3F202	adequate	3	+
3F203	adequate	3	+
3F204	adequate	3	+
3F205	adequate	3	+
4F276	adequate	3	+
4F277	adequate	3	+
4F278	adequate	3	+
4F279	adequate	3	+
4F280	adequate	2 - 3	+
5F351	adequate	2 - 3	+
5F352	adequate	2 - 3	+
5F353	adequate	2 - 3	+
5F354	adequate	3	+
5F355	adequate	3	+
6F426	adequate	2	+
6F427	adequate	2	+
6F428	adequate	2	+
6F429	adequate	2	+
6F430	adequate	2	+

Abbreviations: Fe = iron; ID = identification

5.0 DISCUSSION AND CONCLUSIONS

The objective of this study was to evaluate the toxicity and potential mechanisms of action of sodium dichromate dehydrate (SDD) administered in drinking water to rats for 90 days. A study

by the National Toxicology Program in which SDD was administered to F344/N rats for 2 years by drinking water demonstrated SDD treatment-related decreases in mean cell volume, mean corpuscular hemoglobin, and presence of anemia with as low as 57.3 mg/L of SDD administration, and mean corpuscular hemoglobin concentration (MCHC) decreases in the 172 and 516 mg/L SDD administration groups.[(2)] The decrease in MCHC and the anemia peaked on Day 22 (MCHC and anemia) or at Month 3 (MCHC) and resolved with time.[(2)] Hemograms were not performed in the current study. However, serum was collected for serum iron measurements and bone marrow smears were stained with Prussian blue stain to assess iron content/storage.

Female Fischer rats administered SDD exhibited a lower mean serum iron level relative to the control group mean at dosages of 60 mg/L or greater. However, the decreases were statistically significant only at the 170 or 520 mg/L SDD dose levels. A few individuals with 60-520 mg/L SDD administration had serum iron decreases that ranged from minimal to marked relative to the control group mean. Additionally, when the data was established against the 2.5-97.5% reference range established from the serum iron levels from the ten control rats, 3/10, 2/10, and 3/10 rats had decreased serum iron levels with administration of 60, 170, and 520 mg/L SDD, respectively. A distinct dose-related relationship between SDD administration and severity of serum iron level decrease, however, was not detected. In conjunction with this, the semi-quantitative assessment of iron content based upon bone marrow smears from 5/5 rats at Day 90 (11/22/2010) suggested decreased iron storage in the bone marrow that was observed in the 170 mg/L SDD group and appeared to be enhanced in the 520 mg/L SDD administration group. Together the results were supportive of decreased serum iron levels and decreased iron storage in the bone marrow of female Fischer rats with 90-day administration of 170 or 520 mg/L SDD. These changes are indicative of a disturbance in iron metabolism, and more specifically this pattern would be expected with iron deficiency.

6.0 REFERENCES

1. Gale E, Torrance J, and Bothwell T. (1963). The quantitative estimation of total iron stores in human bone marrow. *J Clin Invest.* **42**, 1076-82.

2. National Toxicology Program (NTP) (2008). NTP Technical Report on the Toxicology and Carcinogenesis Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies). NTP TR 546. NIH Publication No. 08-5887. National Institutes of Health.

Table I1

Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Iron: Set 1

Day: 91 relative to Start Date

Group	Sex	Animal	Iron ug/dL
1	f	16	374
		17	306
		18	309
		19	297
		20	327
		Mean	322.6
		S.D.	30.7
		N	5
2	f	91	289
		92	357
		93	334
		94	272
		95	360
		Mean	322.4
		S.D.	40.0
		N	5
3	f	166	283
		167	294*
		168	314
		169	307
		170	321
		Mean	303.8
		S.D.	15.3
		N	5

* = Result to left has an associated comment or marker.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table I1

Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Iron: Set 1

Day: 91 relative to Start Date

Group	Sex	Animal	Iron ug/dL
4	f	241	354*
		242	184*
		243	370
		244	188
		245	289
		Mean	277.0
		S.D.	88.4
		N	5
5	f	316	203
		317	191
		318	235
		319	283
		320	311
		Mean	244.6
		S.D.	51.4
		N	5
6	f	391	289
		392	202
		393	247
		394	250
		395	310
		Mean	259.6
		S.D.	41.8
		N	5

* = Result to left has an associated comment or marker.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table I1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Iron: Set 2

Day: 91 relative to Start Date

Group	Sex	Animal	Iron ug/dL
1	f	51	215
		52	340
		53	279
		54	376
		55	334
		Mean	308.8
		S.D.	62.9
		N	5
2	f	126	431
		127	356
		128	412
		129	477
		130	421
		Mean	419.4
		S.D.	43.4
		N	5
3	f	201	349
		202	287
		203	277
		204	336
		205	353
		Mean	320.4
		S.D.	35.8
		N	5

* = Result to left has an associated comment or marker.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table I1

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Iron: Set 2

Day: 91 relative to Start Date

Group	Sex	Animal	Iron ug/dL
4	f	276	326
		277	199
		278	261
		279	249
		280	243
		Mean	255.6
		S.D.	45.8
		N	5
5	f	351	306
		352	230
		353	290
		354	230
		355	252
		Mean	261.6
		S.D.	34.9
		N	5
6	f	426	275
		427	222
		428	235
		429	266
		430	218
		Mean	243.2
		S.D.	25.9
		N	5

* = Result to left has an associated comment or marker.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table I1

Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Individual Serum Iron

Day	Group	Sex	Animal	Measurement	Comments and Markers: Type	Marker	Comment
91	3	f	167	Iron	Result		Sample diluted 1:1 to obtain this value
	4	f	241	Iron	Result		Sample diluted 1:1 to obtain this value
			242	Iron	Result		Sample diluted 1:1 to obtain this value

* = Result to left has an associated comment or marker.

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Table I2

90-Day Repeat Dose Toxicity Study of Sodium Dichromate Dihydrate
Administered in Drinking Water to Fischer Rats

Summary of Serum Iron

Day: 91 relative to Start Date

Group	Sex		Iron ug/dL
1	f	Mean	315.7
		S.D.	47.2
		N	10
2	f	Mean	370.9
		S.D.	64.5
		N	10
3	f	Mean	312.1
		S.D.	27.4
		N	10
4	f	Mean	266.3
		S.D.	67.4
		N	10
5	f	Mean	253.1*
		S.D.	42.4
		N	10
6	f	Mean	251.4*
		S.D.	33.9
		N	10

Statistics Test: One-way ANOVA run with 5% significance; post hoc Dunnett Test: * - 5% significance level (Groups 2-6 to Group 1)

Nominal Dose: Group 1 - 0 mg/L Water Group 2 - 0.3 mg/L SDD Group 3 - 4 mg/L SDD
Group 4 - 60 mg/L SDD Group 5 - 170 mg/L SDD Group 6 - 520 mg/L SDD

Appendix J

Statistics Contributing Scientist Report

Statistical Report for 13026.01.02 Water Intake, Food Intake, Body Weight, and Other Endpoints

FINAL

Southern Research Institute Project 13026.01.02

Submitted by:

Nicola Richardson-Harman Ph.D.

Statistical Consultant

Alpha StatConsult LLC.

www.alphastatconsult.com

February 11th 2011

SIGNATURE PAGE

Statistical Report for 13026.01.02 Water Intake, Food Intake, Body Weight, and Other Endpoints

FINAL

2-11-2011

Name and credentials of statistician

Date

Nicola Richardson-Harman Ph.D.

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Contents

1.0 Objectives

To compare, between treatment groups 2-6 and placebo group 1:

- daily food intake, per cage (g/rat/day).
- daily water intake, per cage (mL/rat/day).
- body weight (g), per animal.
- a series of bio-measures.

2.0 Data

Food intake (g/rat/day) were provided for 13 time points: Days 1-8, 8-15, 15-22, 22-29, 29-36, 36-43, 43-50, 50-57, 57-64, 64-71, 71-78, 78-85, and 85-91.

Water intake (mL/rat/day) were provided for 13 time points: Days 1-8, 8-15, 15-22, 22-29, 29-36, 36-43, 43-50, 50-57, 57-64, 64-71, 71-78, 78-85, and 85-91.

Body weight (g) data were provided for 15 time points: Week-1, and Days 1, 8, 15, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85, and 91.

The following bio-measures were provided, per animal:

- 8-Isoprostane in Duodenum (ng/mL) on Day 91.
- 8-Isoprostane in Oral Cavity (ng/mL) on Day 91.
- 8-OHdG in Duodenum (ng/mL) on Day 91.
- 8-OHdG in Oral Cavity (ng/mL) on Day 91.
- Ferritin Levels (ng/mL) on Day 91.
- Iron (μg/dL) on Day 91.
- Transferrin Levels (mg/mL) on Day 91.

3.0 Statistical Methods

The Kolmogorov-Smirnov statistic (alpha = 0.001) was used to test whether the data were normally distributed.

For normally distributed data a one-way Analysis of Variance (alpha = 0.05) was performed at each time point followed by Dunnett's post hoc comparison (alpha = 0.05) to compare groups 2-6 to control group 1.

Data with unequal variance between groups were $\log_{10}$ transformed prior to statistical analyses. SAS™ Version 9.2 and an alpha = 0.05 was used for all inter-group comparisons.

4.0 Results

Food Intake

The food intake data met criteria for normality. The results of the one way ANOVA across groups (groups 1-6) and Dunnett's post hoc test between groups 2-6 and the control group 1, are given in Table 1 a&b. Food intake was higher for animals in group 6, compared to the control at day 43-50 and 57-64 (Table 1b).

Water Intake

The water intake data met criteria for normality. The results of the one way ANOVA across groups (groups 1-6) and Dunnett's post hoc test between groups 2-6 and the control group 1, are given in Table 2 a&b. Water intake was lower for group 4, compared to the control at Days 22-50, 71-78 and 85-91 (Table 2a). Water intake was lower for animals in groups 5 and 6, compared to the control, for all days tested (Table 2b).

Body Weight

Body weight data met criteria for normality. The results of the one way ANOVA across groups (groups 1-6) and Dunnett's post hoc test between groups 2-6 and the control group 1, are given in Table 3 a&b. There was a significant effect of treatment at Days 22 and 50-71 that was not due

to a difference between treatment groups 2-6 and the control (Table 3a&b). On post-hoc analysis, body weight was lower for group 5 animals at Day 78 and for group 6 animals at Days 36, 43, 85, and 91 (Table 3b).

Bio Measures

Measurements of 8-Isoprostane in Oral Cavity were found have unequal variance between groups and were $\log_{10}$ transformed prior to statistical analyses. All bio measures met criteria for normality. The results of the one way ANOVA across bio measures for groups (groups 1-6) and Dunnett's post hoc test between groups 2-6 and the control group 1, are given in Tables 4-10. Levels of 8-OHdG in Duodenum were lower for animals in groups 5 and 6 compared to the placebo group (Table 6). Levels of Ferritin were higher in groups 2 and 4 compared to the placebo (Table 8). Iron levels were lower in groups 5 and 6 compared to the placebo (Table 9). There was a significant effect of treatment for Log_{10} [8-Isoprostane in Oral Cavity] but on post-hoc analysis there were no statistically significant differences between the treatment groups and the placebo (Table 5). There were no significant differences between groups in measurements of 8-Isoprostane in Duodenum (Table 4), 8-OHdG in Oral Cavity (Table 7), and Transferrin (Table 10).

Tables

Table 1. Food Intake Per Cage (g/rat/day) Days 1-91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with: (a) Groups 2-4; (b) Groups 5-6. Group means (standard deviation) and number of animals per group are given.

a.

Days	Pr > F	Prob.	Group 1	Group 2	D_2	Group 3	D_3	Group 4	D_4
1-8	0.1961	ns	10.15 (0.98) n=15	10.26 (0.9) n=14	ns	10.04 (0.83) n=14	ns	10.16 (1.15) n=13	ns
8-15	0.7870	ns	10.61 (1.61) n=10	10.97 (1.43) n=10	ns	10.55 (1.88) n=10	ns	10.98 (1.19) n=9	ns
15-22	0.1065	ns	11.14 (0.75) n=10	11.02 (0.97) n=10	ns	10.89 (0.81) n=10	ns	10.26 (1.06) n=10	ns
22-29	0.9388	ns	10.48 (1.07) n=9	10.61 (1.05) n=10	ns	10.37 (0.83) n=8	ns	10.47 (0.72) n=10	ns
29-36	0.1266	ns	10.14 (0.84) n=10	10.15 (0.23) n=10	ns	10.23 (0.43) n=9	ns	10.31 (0.3) n=10	ns
36-43	0.3288	ns	10.13 (0.47) n=10	10.12 (0.53) n=10	ns	9.89 (0.88) n=10	ns	10.52 (1.18) n=10	ns
43-50	0.0158	<0.05	9.47 (0.87) n=10	9.75 (0.22) n=10	ns	9.71 (0.4) n=10	ns	10.01 (0.49) n=10	ns
50-57	0.4538	ns	9.95 (0.57) n=10	9.8 (0.6) n=10	ns	9.89 (0.39) n=10	ns	9.96 (0.48) n=10	ns
57-64	0.0015	<0.01	9.9 (0.48) n=10	9.94 (0.5) n=10	ns	9.8 (0.43) n=10	ns	9.92 (0.37) n=9	ns
64-71	0.5575	ns	9.71 (0.35) n=9	9.87 (0.49) n=10	ns	9.82 (0.47) n=10	ns	9.96 (0.71) n=9	ns
71-78	0.3223	ns	9.62 (0.42) n=10	9.59 (0.49) n=10	ns	9.61 (0.49) n=10	ns	9.58 (0.42) n=10	ns
78-85	0.9991	ns	9.76 (0.59) n=10	9.77 (0.67) n=10	ns	9.74 (0.64) n=10	ns	9.79 (0.52) n=10	ns
85-91	0.2820	ns	9.89 (0.39) n=10	9.95 (0.52) n=10	ns	9.32 (1.28) n=10	ns	9.88 (0.88) n=7	ns

Where: Pr>F = ANOVA Probability of no difference between groups 1-6; Prob. = ANOVA Probability level; D_n = Dunnett's test result where Group n is compared to placebo Group 1; * Dunnett's $P<0.05$; ns = non-significant at $P<0.05$.

Table 1. continued.

b.

Days	Pr > F	Prob.	Group 1	Group 5	D_5	Group 6	D_6
1-8	0.1961	ns	10.15 (0.98) n=15	9.67 (0.79) n=14	ns	9.48 (0.82) n=12	ns
8-15	0.7870	ns	10.61 (1.61) n=10	10.3 (1.67) n=10	ns	11.18 (0.6) n=9	ns
15-22	0.1065	ns	11.14 (0.75) n=10	10.47 (1.13) n=10	ns	11.74 (1.96) n=9	ns
22-29	0.9388	ns	10.48 (1.07) n=9	10.59 (1.27) n=10	ns	10.82 (0.57) n=10	ns
29-36	0.1266	ns	10.14 (0.84) n=10	10.28 (0.46) n=10	ns	10.96 (1.33) n=9	ns
36-43	0.3288	ns	10.13 (0.47) n=10	9.97 (0.43) n=10	ns	10.4 (0.41) n=10	ns
43-50	0.0158	<0.05	9.47 (0.87) n=10	9.92 (0.68) n=10	ns	10.43 (0.54) n=9	*
50-57	0.4538	ns	9.95 (0.57) n=10	9.75 (0.3) n=10	ns	10.18 (0.44) n=9	ns
57-64	0.0015	<0.01	9.9 (0.48) n=10	9.83 (0.43) n=9	ns	10.65 (0.58) n=10	*
64-71	0.5575	ns	9.71 (0.35) n=9	9.76 (0.6) n=10	ns	10.16 (0.44) n=8	ns
71-78	0.3223	ns	9.62 (0.42) n=10	9.51 (0.48) n=9	ns	9.98 (0.45) n=9	ns
78-85	0.9991	ns	9.76 (0.59) n=10	9.84 (0.33) n=9	ns	9.83 (0.96) n=9	ns
85-91	0.2820	ns	9.89 (0.39) n=10	9.82 (0.57) n=9	ns	10.17 (0.55) n=8	ns

Where: Pr>F = ANOVA Probability of no difference between groups 1-6; Prob. = ANOVA Probability level; D_n = Dunnett's test result where Group *n* is compared to placebo Group 1; * Dunnett's $P<0.05$; ns = non-significant at $P<0.05$

Table 2. Water Intake Per Cage (mL/rat/day) Days 1-91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with: (a) Groups 2-4; (b) Groups 5-6. Group means (standard deviation) and number of animals per group are given.

a.

Days	Pr > F	Prob.	Group 1	Group 2	D_2	Group 3	D_3	Group 4	D_4
1-8	<.0001	<0.001	23.63 (1.4) n=14	24.59 (1.59) n=15	ns	23.72 (2.07) n=15	ns	23.41 (2) n=15	ns
8-15	<.0001	<0.001	23.91 (2.69) n=9	23.95 (2.28) n=10	ns	23.31 (3.33) n=10	ns	22.81 (2.19) n=10	ns
15-22	0.0001	<0.001	24.48 (1.44) n=10	23.67 (1.53) n=10	ns	25.01 (1.48) n=9	ns	25.53 (10.39) n=9	ns
22-29	<.0001	<0.001	25.01 (5.37) n=10	22.72 (1.27) n=10	ns	24.34 (3.31) n=10	ns	21.57 (1.05) n=10	*
29-36	<.0001	<0.001	23.83 (1.04) n=10	22.92 (1.49) n=10	ns	23.35 (1.43) n=10	ns	21.53 (1.25) n=10	*
36-43	<.0001	<0.001	23.7 (2.76) n=10	22.34 (0.97) n=10	ns	22.57 (1.65) n=10	ns	20.22 (0.96) n=10	*
43-50	<.0001	<0.001	22.92 (3.8) n=10	21.6 (1.64) n=10	ns	21.77 (1.53) n=10	ns	20.17 (0.94) n=9	*
50-57	<.0001	<0.001	22.22 (2.04) n=10	23.4 (4.81) n=9	ns	22.58 (1.6) n=10	ns	19.81 (0.82) n=9	ns
57-64	<.0001	<0.001	21.68 (1.63) n=10	21.91 (1.92) n=10	ns	22.05 (1.8) n=10	ns	19.89 (1.2) n=10	ns
64-71	<.0001	<0.001	21.28 (1.62) n=10	21.47 (1.54) n=10	ns	21.9 (1.84) n=10	ns	19.44 (1.17) n=10	ns
71-78	<.0001	<0.001	20.81 (1.04) n=10	20.31 (1.98) n=10	ns	20.94 (1.53) n=9	ns	18.97 (1.52) n=10	*
78-85	<.0001	<0.001	21.02 (1.27) n=9	20.8 (1.73) n=10	ns	21.62 (1.33) n=10	ns	19.31 (0.88) n=10	ns
85-91	<.0001	<0.001	20.74 (0.69) n=10	20.32 (1.2) n=9	ns	20.64 (1.17) n=8	ns	18.32 (0.77) n=10	*

Where: Pr>F = ANOVA Probability of no difference between groups 1-6; Prob. = ANOVA Probability level; D_n = Dunnett's test result where Group *n* is compared to placebo Group 1; * Dunnett's $P<0.05$; ns = non-significant at $P<0.05$.

Table 2. continued.

b.

Days	Pr > F	Prob.	Group 1	Group 5	D_5	Group 6	D_6
1-8	<.0001	<0.001	23.63 (1.4) n=14	20.15 (2.32) n=15	*	17.7 (4.22) n=15	*
8-15	<.0001	<0.001	23.91 (2.69) n=9	18.79 (2.58) n=10	*	16.81 (1.5) n=10	*
15-22	0.0001	<0.001	24.48 (1.44) n=10	19.49 (1.35) n=10	*	17.2 (1.12) n=10	*
22-29	<.0001	<0.001	25.01 (5.37) n=10	18.43 (1.18) n=10	*	17.78 (2.87) n=10	*
29-36	<.0001	<0.001	23.83 (1.04) n=10	18.21 (1.05) n=10	*	18.28 (3.21) n=10	*
36-43	<.0001	<0.001	23.7 (2.76) n=10	17.82 (0.8) n=10	*	16.27 (0.99) n=10	*
43-50	<.0001	<0.001	22.92 (3.8) n=10	17.41 (0.73) n=10	*	16.21 (0.85) n=9	*
50-57	<.0001	<0.001	22.22 (2.04) n=10	17.21 (0.84) n=10	*	16.31 (1.06) n=10	*
57-64	<.0001	<0.001	21.68 (1.63) n=10	17.08 (1.21) n=10	*	17.1 (3.58) n=10	*
64-71	<.0001	<0.001	21.28 (1.62) n=10	16.43 (0.72) n=10	*	16.8 (4.03) n=10	*
71-78	<.0001	<0.001	20.81 (1.04) n=10	16.02 (0.57) n=9	*	15.38 (1.39) n=9	*
78-85	<.0001	<0.001	21.02 (1.27) n=9	17.26 (1.92) n=10	*	14.89 (1.37) n=10	*
85-91	<.0001	<0.001	20.74 (0.69) n=10	16.73 (1.34) n=10	*	15.51 (2.48) n=10	*

Where: Pr>F = ANOVA Probability of no difference between groups 1-6; Prob. = ANOVA Probability level; D_n = Dunnett's test result where Group *n* is compared to placebo Group 1; * Dunnett's $P<0.05$; ns = non-significant at $P<0.05$.

Table 3. Animal Body Weight (g) Week-1 to Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with: (a) Groups 2-4; (b) Groups 5-6. Group means (standard deviation) and number of animals per group are given.

a.

Day	Pr > F	Prob.	Group 1	Group 2	D_2	Group 3	D_3	Group 4	D_4
Week -1	1.0000	ns	82.84 (7.48) n=75	82.85 (7.5) n=75	ns	82.86 (7.51) n=75	ns	82.83 (7.47) n=75	ns
1	0.9928	ns	104.89 (8.32) n=75	105.34 (9.09) n=75	ns	105.53 (7.74) n=75	ns	105.14 (9.36) n=75	ns
8	0.7604	ns	115.32 (8.74) n=75	115.49 (10.3) n=75	ns	115.41 (9.15) n=75	ns	116 (10.76) n=75	ns
15	0.3842	ns	123.57 (11.04) n=50	124.46 (11.59) n=50	ns	123.31 (13.09) n=50	ns	127.01 (9.52) n=50	ns
22	0.0465	<0.05	138.61 (6.24) n=50	138.04 (6.91) n=50	ns	138.86 (6.66) n=50	ns	138.46 (5.65) n=50	ns
29	0.2029	ns	145.45 (6.04) n=50	145.18 (7.57) n=50	ns	144.85 (6.37) n=50	ns	145.8 (5.58) n=50	ns
36	0.0242	<0.05	150.65 (5.67) n=50	149.29 (7.58) n=50	ns	150.94 (6.29) n=50	ns	150.65 (5.18) n=50	ns
43	0.0026	<0.01	154.57 (6.25) n=50	154.03 (8.24) n=50	ns	155.44 (6) n=50	ns	154.62 (5.93) n=50	ns
50	0.0125	<0.05	155.97 (9.53) n=50	157.87 (7.61) n=50	ns	159.05 (7.09) n=50	ns	158.6 (6.25) n=50	ns
57	0.0080	<0.01	161.32 (7.39) n=50	160.76 (8.07) n=50	ns	162.94 (7.12) n=50	ns	162.34 (6.68) n=50	ns
64	0.0132	<0.05	163.5 (6.93) n=50	163.15 (8.17) n=50	ns	164.94 (7.18) n=50	ns	164.93 (6.45) n=50	ns
71	0.0028	<0.01	168.33 (6.75) n=50	167.81 (9.15) n=50	ns	170.34 (7.88) n=50	ns	169.04 (6.14) n=50	ns
78	0.0004	<0.001	169.53 (7.33) n=50	168.87 (8.83) n=50	ns	170.85 (7.65) n=50	ns	170.03 (6.13) n=50	ns
85	0.0019	<0.01	173.06 (7.07) n=50	172.68 (9.23) n=50	ns	174.58 (8.07) n=50	ns	174.27 (6.48) n=50	ns
91	0.0043	<0.01	177.81 (7.44) n=50	176.73 (9.26) n=50	ns	176.36 (10.85) n=50	ns	177.23 (6.75) n=50	ns

Where: Pr>F = ANOVA Probability of no difference between groups 1-6; Prob. = ANOVA Probability level; D_n = Dunnett's test result where Group n is compared to placebo Group 1; * Dunnett's $P<0.05$; ns = non-significant at $P<0.05$.

Table 3. continued.

b.

Day	Pr > F	Prob.	Group 1	Group 5	D_5	Group 6	D_6
Week -1	1.0000	ns	82.84 (7.48) n=75	82.82 (7.44) n=75	ns	82.89 (7.49) n=75	ns
1	0.9928	ns	104.89 (8.32) n=75	105.63 (8.15) n=75	ns	104.93 (8.75) n=75	ns
8	0.7604	ns	115.32 (8.74) n=75	114.88 (7.89) n=75	ns	113.75 (8.31) n=75	ns
15	0.3842	ns	123.57 (11.04) n=50	122.69 (11.19) n=50	ns	123.09 (7.96) n=50	ns
22	0.0465	<0.05	138.61 (6.24) n=50	136.54 (6.4) n=50	ns	135.39 (6.84) n=50	ns
29	0.2029	ns	145.45 (6.04) n=50	143.73 (6.82) n=50	ns	142.82 (7.37) n=50	ns
36	0.0242	<0.05	150.65 (5.67) n=50	148.28 (7) n=50	ns	147.24 (7.38) n=50	*
43	0.0026	<0.01	154.57 (6.25) n=50	151.99 (6.79) n=50	ns	150.54 (7.4) n=50	*
50	0.0125	<0.05	155.97 (9.53) n=50	155.99 (6.62) n=50	ns	154.36 (7.26) n=50	ns
57	0.0080	<0.01	161.32 (7.39) n=50	159.14 (7.7) n=50	ns	157.92 (8.31) n=50	ns
64	0.0132	<0.05	163.5 (6.93) n=50	161.6 (7.32) n=50	ns	160.5 (7.93) n=50	ns
71	0.0028	<0.01	168.33 (6.75) n=50	165.64 (7.93) n=50	ns	164.69 (8.38) n=50	ns
78	0.0004	<0.001	169.53 (7.33) n=50	164.67 (9.33) n=50	*	165.82 (8.85) n=50	ns
85	0.0019	<0.01	173.06 (7.07) n=50	170.42 (8.1) n=50	ns	168.78 (9.35) n=50	*
91	0.0043	<0.01	177.81 (7.44) n=50	173.73 (8.13) n=50	ns	171.93 (8.92) n=50	*

Where: Pr>F = ANOVA Probability of no difference between groups 1-6; Prob. = ANOVA Probability level; D_n = Dunnett's test result where Group *n* is compared to placebo Group 1; * Dunnett's $P<0.05$; ns = non-significant at $P<0.05$.

Table 4. 8-Isoprostane Duodenum (ng/mL) at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	8-Isoprostane Duodenum (ng/mL)	Dunnett's[α]
1	208.03 (235.63) n=5	
2	207.52 (248.24) n=5	ns
3	269.9 (129.21) n=5	ns
4	236.15 (238.21) n=5	ns
5	712.89 (812.34) n=5	ns
6	109.45 (218.51) n=5	ns
ANOVA Probability	0.2195	
Significance	ns	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
ns = non significant

Table 5. Log_{10}[8-Isoprostane Oral Cavity]ng/mL at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	Log_{10} [8-Isoprostane Oral Cavity] ng/mL	Dunnett's[α]
1	1.89 (0.49) n=5	
2	1 (0.36) n=5	ns
3	2.13 (1.03) n=5	ns
4	2.54 (1.05) n=5	ns
5	2.19 (0.57) n=5	ns
6	1.81 (0.29) n=5	ns
ANOVA Probability	0.04	
Significance	<0.05	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
ns = non significant

Table 6. 8-OHdG Duodenum (ng/mL) at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	8-OHdG Duodenum (ng/mL)	Dunnett's[α]
1	134.78 (11.89) n=5	
2	134.11 (16.9) n=5	ns
3	134.65 (16.09) n=5	ns
4	103.64 (19.47) n=5	ns
5	90.43 (29.5) n=5	*
6	80.51 (30.42) n=5	*
ANOVA Probability	0.0007	
Significance	<0.001	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
*Dunnett's $P<0.05$
ns = non significant

Table 7. 8-OHdG Oral Cavity (ng/mL) at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	8-OHdG Oral Cavity (ng/mL)	Dunnett's[α]
1	59.78 (39.1) n=5	
2	61.76 (38.77) n=5	ns
3	57.59 (25.31) n=5	ns
4	50.2 (44.05) n=5	ns
5	42.79 (12.98) n=5	ns
6	63.69 (37.63) n=5	ns
ANOVA Probability	0.9281	
Significance	ns	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
ns = non significant

Table 8. Ferritin Levels (ng/mL) at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	Ferritin Levels (ng/mL)	Dunnett's[α]
1	885.4 (223.43) n=5	
2	1546.8 (315.01) n=5	*
3	1003.8 (153.23) n=5	ns
4	1529.2 (354.87) n=5	*
5	1313.8 (437.45) n=5	ns
6	1250 (449.57) n=5	ns
ANOVA Probability	0.0251	
Significance	<0.05	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
*Dunnett's $P<0.05$
ns = non significant

Table 9. Iron Levels (µg/dL) at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	Iron (µg/dL)	Dunnett's[α]
1	315.7 (47.22) n=10	
2	370.9 (64.51) n=10	ns
3	312.1 (27.39) n=10	ns
4	266.3 (67.35) n=10	ns
5	253.1 (42.39) n=10	*
6	251.4 (33.88) n=10	*
ANOVA Probability	<0.001	
Significance	<0.001	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
*Dunnett's $P<0.05$
ns = non significant

Table 10. Transferrin Levels (mg/mL) at Day 91.

ANOVA and Dunnett's test results to compare Group 1 (placebo) with Groups 2-6. Group means (standard deviation) and number of animals per group are given.

Group	Transferrin Levels (mg/mL)	Dunnett's[α]
1	3.06 (0.41) n=5	
2	3.31 (0.33) n=5	ns
3	2.93 (0.37) n=5	ns
4	2.63 (0.2) n=5	ns
5	2.67 (0.37) n=5	ns
6	2.51 (0.24) n=5	ns
ANOVA Probability	0.0067	
Significance	<0.01	

[α]Dunnett's comparison between Group 1 (placebo) and groups 2-6.
ns = non significant