

# **I. Protocol Outline of the NTP 13-Week Toxicity Studies of Sodium Dichromate Dihydrate (Drinking Water Studies)**

CAS Number: 7789-12-0  
Molecular Formula:  $\text{Na}_2\text{Cr}_2\text{O}_7 \cdot 2\text{H}_2\text{O}$   
Molecular Weight: 298

## **OBJECTIVES:**

To characterize the toxicity of chromium VI (Cr VI) given in the drinking water as sodium dichromate dihydrate, to delineate differences in response between sexes and species, and to select the appropriate doses for use in the 2-year carcinogenicity studies.

## **SOURCE OF NOMINATION AND RATIONALE FOR TESTING:**

California Senator Schiff, California EPA Senior officials, and California Health & Human Services Department nominated Cr VI in drinking water for testing by the NTP. Reasons for the nomination; a) inadequacy of existing long-term animal studies by the oral route; b) recent findings of measurable Cr VI, a known human carcinogen when inhaled, in water of several California cities; c) need for data on gastrointestinal absorption of Cr during the chronic study.

Excellent reviews on the toxicology of chromium can be found in:

- 1) ATSDR publication entitled "Toxicological profile for Chromium". Published in September, 2000 Agency for Toxic Substances & Disease Registry (ATSDR), Division of Toxicology/ Toxicology Information Branch, 1600 Clifton Road NE, E-29, Atlanta Georgia.
- 2) Public Health Goal for Chromium in Drinking Water. February, 1999 document prepared by the Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

## STUDY DESIGN

### 1. TREATMENT

After a ten to fourteen day quarantine period, groups of both sexes of F344 rats and B6C3F<sub>1</sub> mice shall be assigned at random to treatment and control groups. At each of five doses plus a control group (0, 62.5, 125, 250, 500 or 1000 mg/L equivalent to 0, 24, 48, 96, 192, or 384 mg Cr VI /L), ten animals per sex per species shall receive the test chemical in drinking water for 90 days after which they shall be sacrificed with no recovery period. Additional rats (both sexes) shall be treated at the same doses for conducting special studies. Male mice shall be housed individually. Male and female rats and female mice shall be housed five per cage.

	<u>Animals</u>	<u>Species</u>	<u>Sexes</u>	<u>Test</u> <u>Groups</u>	<u>Total</u>
<u>Core Study</u>					
Treatment	10	x	2 x 2	x 5	= 200
Controls	10	x	2 x 2	x 1	= 40
<u>Special Study</u>					
Treatment	10	x	1 x 2	x 5	= 100
Controls	10	x	1 x 2	x 1	= 20
Total					<u>360</u>

### 2. OBSERVATIONS

Core animals shall be weighed individually on day one on test, weekly and at necropsy. Water consumption shall be recorded (by cage) over a 7-day period, at weekly periods. All animals shall be observed twice daily, once in the early morning and once in the late afternoon, at least six hours apart (before 10:00 AM and after 2:00 PM), including holidays and weekends for signs of moribundity and death. Signs of toxicity noticed during these routine checks for core animals shall be recorded. Clinical observations shall be performed and recorded weekly for core study animals. For the special study animals, body weights, clinical observations and water consumption are not required.

### 3. SPECIAL STUDIES

#### a. CLINICAL LABORATORY STUDIES

Special study rats of both sexes are to be treated at the same doses as the core study for use in conducting clinical laboratory studies. Male and female rats shall have blood collected from the retro-orbital sinus under CO<sub>2</sub> anesthesia for the conduct of clinical lab studies on days 4 ± 1, and 21 ± 2. All male and female animals shall be treated the same number of days before collection of samples and all animals of a sex shall be bled on the same day. The special clinical lab study animals shall be used for urinalysis (24-hour urine collection) on day 14 ± 2. During the urine collection period, animals are to be provided feed and treated water ad libitum. Clinical lab studies shall also be conducted on core study rats at terminal sacrifice. Hematology measurements shall be made on core study mice at terminal sacrifice.

#### Hematology

Erythrocyte count  
Mean corpuscular volume  
Hemoglobin  
Packed cell volume  
Mean corpuscular hemoglobin  
Mean corpuscular hemoglobin concentration  
Erythrocyte morphologic assessment  
Leukocyte count  
Leukocyte differential  
Reticulocyte count  
Platelet count and morphologic assessment

#### Clinical Chemistries (Listed in priority order)

Alanine aminotransferase (ALT)  
Sorbitol dehydrogenase (SDH)  
Total bile acids  
Alkaline phosphatase (ALP)  
Urea Nitrogen (BUN)  
Creatinine  
Total protein  
Albumin  
Glucose  
Creatine kinase (CK)  
Sodium  
Potassium  
5"-Nucleotidase  
Cholesterol  
Calcium  
Phosphate  
Triglycerides

**Urinalysis**  
**Urine volume**  
**Specific gravity**  
**pH**  
**Creatinine**  
**AST**  
**ALP**  
**NAG**  
**Glucose**  
**Protein**

Earlier reports indicated that chromium administered orally caused liver toxicity (hepatocyte cytoplasmic vacuolization as well as kidney toxicity (renal tubular necrosis). Accordingly, specific tests used as early indicators of such toxicities were included in the design.

**b. BLOOD SMEARS FOR MICRONUCLEI**

Two unstained blood smears shall be prepared from mice at termination of the 90-day study for use by the NTP in micronuclei determinations. This test is used for identifying DNA damage that could be attributed to chemical administration.

**4. NECROPSY AND PATHOLOGY**

Organ weights shall be determined from all core study animals surviving until the end of the study. Those organs to be weighed are: liver, spleen, thymus, right kidney, right testis, heart, and lungs. Organs shall be weighed to the nearest 10.0 mg except for testis and thymus which shall be weighed to the nearest 1.0 mg.

A complete necropsy shall be performed on all core study treated and control animals that either die or are sacrificed, and all tissues as listed below shall be saved in formalin. All tissues required for complete histopathology shall then be trimmed, embedded, sectioned and stained with hematoxylin and eosin for possible histopathologic evaluation. This shall be done for all animals in all groups.

Gross lesions shall be examined in all animals in all dose groups plus controls. A complete histopathologic evaluation shall be done on all core control animals, all core animals in the highest dose group with at least 60% survivors at the time of sacrifice, plus all core animals in higher dose groups. Chemical-related lesions (target tissues) shall be identified, and these tissues shall be examined in lower dose groups to a no-effect-level. For all natural death/moribund sacrifice animals, a complete histopathologic evaluation shall be performed.

The following revised protocol took into consideration the recommendations of the July 2002 NTP Expert Panel on Chromium

## II. Two-Year Toxicity and Carcinogenicity Studies of **Sodium Dichromate Dihydrate in F344 Rats and B6C3F<sub>1</sub> Mice (Drinking Water Study)**

### 1. OBJECTIVES

To determine the chronic toxicity and carcinogenicity as well as the toxicokinetics of sodium dichromate dihydrate given to rats and mice in their drinking water.

### 2. TREATMENT

After a ten to fourteen day quarantine period, all animals shall be assigned at random to treatment and control groups. Female rats and female mice shall be housed five animals per cage, and male rats shall be housed three per cage. Male mice shall be individually housed.

Rats and mice shall receive the test chemical for 104 weeks in tap water at 4 doses plus control. (rats and female mice: 0, 14.3, 57.3, 172, or 516 mg/L; male mice: 0, 14.3, 28.6, 85.7, or 258 mg/L).

	<u>Animals</u>		<u>Species</u>		<u>Sexes</u>		<u>Test Groups</u>		<u>Total</u>
<u>Core Study</u>									
Treatment	50	x	2	x	2	x	4	=	800
Controls	50	x	2	x	2	x	1	=	200
Sentinels	15	x	2	x	2			=	<u>60</u>
									1060
<u>Special Study</u>									
Treatment	40	x	2	x	1	x	4	=	320
Controls	40	x	2	x	1	x	1	=	<u>80</u>
									400

### 3. OBSERVATIONS

All animals shall be observed two times daily for moribundity and mortality including holidays and weekends.

Each core study animal shall be formally examined for clinical signs of toxicity at four-week intervals and these observations shall be recorded. Signs of toxicity detected at times other than the formal four-week observations shall be noted and recorded.

Individual animal body weights for treated and control core and special study animals shall be

recorded on day one on test, at weekly intervals for the first 13 weeks, at 4 week intervals thereafter and at terminal sacrifice. If life threatening tumors develop, a significant number of deaths occur, or a significant effect on body weight is observed, the weighing frequency may be increased to every two weeks upon approval by the NTP Project Officer. It is estimated that animals will be weighed every two weeks for the final thirteen weeks of the chronic study. Special study animals shall also be weighed at the time of removal to metabolism cages.

Water consumption shall be recorded (by cage) over a 7-day period, weekly for the first 13 weeks, and every 4 weeks thereafter for core and special study animals. Water consumption shall also be recorded (by cage) for special study animals for the 3 day period prior to their removal on days 4 and 11.

#### **4. NECROPSY AND PATHOLOGY**

A complete necropsy shall be performed on all core study treated and control animals that either die or are sacrificed, and all tissues as listed in the Specifications, Section II.H. shall be saved in formalin. All tissues required for complete histopathology, plus the eye and harderian gland, shall then be trimmed, embedded, sectioned and stained with hematoxylin and eosin for possible histopathologic evaluation. This shall be done for all animals in all groups.

Gross lesions shall be examined in all animals in all dose groups plus controls. A complete histopathologic evaluation shall be done on all core control animals, all core animals in the highest dose group with at least 60% survivors at the time of sacrifice, plus all core animals in higher dose groups. Chemical-related lesions (target tissues) shall be identified, and these tissues shall be examined in lower dose groups to a no-effect-level. For all natural death/moribund sacrifice animals, a complete histopathologic evaluation shall be performed.

## LIST OF TISSUES FOR COMPLETE HISTOPATHOLOGIC EVALUATION

Adrenal glands	adjacent skin
Brain (3 sections including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons)	Muscle, thigh (if neuromuscular signs were present)
Clitoral glands	Nasal cavity and nasal turbinates (3 sections)
Esophagus	Ovaries
Eyes (if grossly abnormal)	Pancreas
Femur, including diaphysis with marrow cavity and epiphysis (femoral condyle with epiphyseal cartilage plate, articular cartilage and articular surface)	Parathyroid glands
Gallbladder (mouse)	Pituitary gland
Gross lesions	Preputial glands
Heart and aorta	Prostate
Intestine, large (cecum, colon, rectum)	Salivary glands
Intestine, small (duodenum, jejunum, ileum)	Seminal vesicle
Kidneys	Spinal cord and sciatic nerve (if neurologic signs were present)
Liver (2 sections including left lateral lobe and median lobe)	Spleen
Lungs and mainstem bronchi	Stomach (forestomach and glandular)
Lymph nodes	Testes with epididymus
- mandibular and mesenteric	Thymus
Mammary gland with	Thyroid glands
	Tissue masses and regional lymph nodes
	Trachea
	Urinary bladder
	Uterus

## **5. SPECIAL STUDIES**

### **TOXICOKINETICS**

During the 2-year study, male rats and female mice (10 per exposure group and control) shall be randomly selected from the special study animals and removed from treatment on the morning of day 4, day 11 and after 6, and 12 months treatment. All ten mice, and five rats (randomly selected from the 10) will be placed in metabolism cages with access to untreated water and feed ad libitum. Animals will remain in the same metabolism cages over a 48 hour period, during which time two separate urine and feces samples are to be collected (from 0 - 24 hours, and from 24 - 48 hours). Steps must be taken to keep urine and feces separate. Urine volume and creatinine will be measured and recorded; fecal weight will be recorded. Urine and fecal samples are to be frozen at  $-20^{\circ}\text{C}$  until shipped. The five rats not selected for metabolism studies are to be housed in the same manner as special study animals during this 48 hour period, except they are to be moved to cages where they are given access ad libitum to untreated water and feed.

At the end of the 48 hour period for each of the 3 time points during the course of the study, all animals selected for the special study (40 male rats or 40 female mice) will be bled and selected tissues taken. Animals are to be bled by heart stick immediately after  $\text{CO}_2$  asphyxiation and 250-500  $\mu\text{l}$  whole blood collected. Following blood collection, each animal will have its abdominal wall opened and arteries severed to allow the animal to bleed out. The entire liver, both kidneys, and stomach (separated into non-glandular and glandular) will be collected and weighed. The time of heparinized blood and tissue collection is to be recorded for each animal. The tissues may remain on ice until collection is complete, after which they will be immediately frozen at  $-20^{\circ}\text{C}$ . Tissue collection from all animals shall be completed within as short a period of time as possible, but must be completed within the same day. Tissues, urine and feces will remain frozen until time of chromium analysis. Heparinized blood samples will be separated into plasma and red blood cell fractions and both fractions will be analyzed for chromium.

### **HEMATOLOGY AND CLINICAL CHEMISTRY**

Hematology and the standard battery of clinical chemistry determinations will be conducted on blood samples collected on days 4, and 21 and at 3, 6, and 12 month from the toxicokinetics study animals