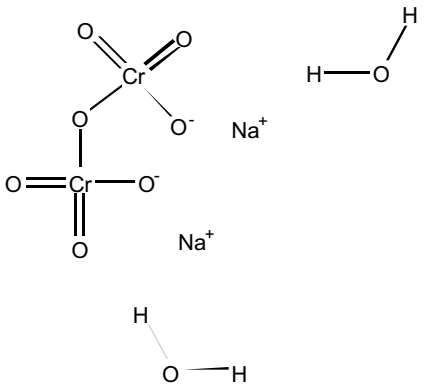
Protocol Outline of the NTP
Comparative 13-Week Toxicity Study of Sodium Dichromate Dihydrate in BALB/C and AM3-C57Bl/6 Transgenic Mice and Evaluation of *in vivo* Mutagenesis of Sodium Dircromate Dihydrate in AM3-C57Bl/6 Mice

CAS Number: 7789-12-0

Chemical Structure:



Molecular formula: $Na_2Cr_2O_{7.2}H_2O$

Molecular weight: 298 g/mol.

Objective:

To determine the toxicity of sodium dichromate dihydrate in BalB/c mice and evaluate the *in vivo* mutagenicity of sodium dichromate dihydrate in am3 transgenic mice (C57/Bl6 mice transgenic for PhiX174am3). The *in vivo* mutagenicity test shall detect reverse mutations of a nonsense codon in the PhiX174 am3 gene.

Source of Nomination and Rationale for Testing:

California EPA requested that NTP study hexavalent chromium toxicity in BalB/C mice because earlier NTP study results indicated compound related hepatotoxicity (hepatic vacuolization) in this strain of mice. Cal EPA further requested that hexavalent chromium be studied in an *in vivo* mutagenicity test other than the micronucleus test. The NTP chose Dr. Heinrich Malling's mutagenicity test which detects reverse mutation of the nonsense codon am3 and forward mutations in gene am3 of phiX174.

Study Design

1. Treatment

After a ten to fourteen day quarantine period, animals shall be assigned at random to treatment and control groups. At each of three concentrations (62.5, 125, or 250 mg/L sodium dichromate dihydrate in drinking-water; plus a control group, 10 male BalB/C mice and 15 male am3-C57Bl/6 transgenic mice per dose group shall receive the subject chemical in drinking water for 13-consecutive weeks. Mice shall be housed individually. Five am3 transgenic mice/group shall be pre-designated for complete necropsy and histopathology. The remainder of the am3 transgenic mice (10/group) shall be used for the *in vivo* mutagenicity assay.

CORE STUDY

	Male Mice		Strain		Test Groups		Total
Treatment	10	x	BalB/C	x	3	=	30
Control	10	x	BalB/C	x	1	=	10
							40
Treatment	5	x	am3	x	3	=	15
Control	5	x	am3	x	1	=	5
							20

MUTAGENICITY ASSAY

	Male Mice		Strain		Test Groups		Total
Treatment	10	x	am3	x	3	=	30
	10	x	am3	x	1	=	10
							40

2. Observations

Animals shall be weighed individually on Day 1 on test, after seven days, and at weekly periods thereafter. The 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 shall be recorded. Formal clinical observations shall be performed and recorded weekly. Water consumption shall be measured weekly.

3. Necropsy and Histopathology of BalB/C Mice and the 5 Pre-designated Transgenic Mice per Group (Core)

A complete necropsy shall be performed on all mice that either die or are sacrificed. Organ weights shall be determined from all 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. After weighing, all tissues as listed in Specifications, Section II.H. shall be saved in neutral buffered formalin. The liver, kidney and forestomach from all these pre-designated mice shall be subjected to histopathology evaluation. These three tissues were selected for histopathology examination because earlier studies suggested that they are potential sites of hexavalent chromium toxicity.

4. Blood Smears for Micronuclei (Core)

Two unstained blood smears shall be prepared from mice at termination of the 90-day study for use by the NTP in micronuclei determinations.

5. Sperm Morphology (Core)

Sperm morphology shall be conducted on mice. The Project Officer shall be notified at the end of the first 70 days of the study regarding mortality, body weight changes and clinical signs of toxicity for use in determining the dose levels to be used in the sperm morphology evaluations.

6. Tissue Collection, and Labeling of 10 Transgenic Mice/Group for *in vivo* Mutagenicity

General Procedure:

All specified tissues shall be placed into pre-labeled eppendorf tubes with color-coded screw tops and O-ringsImmediately after the tissue has been removed from the animal it shall be placed in the color-coded and labeled eppendorf tube and frozen on dry ice. All collected tissues shall be stored at -70°C.

Tissues to be collected , weighed and used in detecting reverse mutation of the nonsense codon am3 and forward mutations in gene am3 of phiX174:

Liver: The total liver is isolated into two 2-ml eppendorf tubes.
Kidney: Each kidney is placed in a separate 1.5-ml eppendorf tube.
Heart: One 1.5-ml eppendorf tube.
Spleen: One 1.5-ml eppendorf tube.
Testis: One testis per1.5-ml eppendorf

Tissues to be isolated for use in detecting reverse mutation of the nonsense codon am3 and forward mutations in gene am3 of phiX174:

Epididymis, cauda, and vas deferens: All samples from one animal are combined into one 1.5-ml eppendorf tube. **Bone marrow:** See enclosed procedure for isolating the bone marrow cells from femur.
Forestomach: One 1.5-ml eppendorf tube per animal. Clean the inside but be careful not to scrape any of the lining off.

Brain: One 1.5-ml eppendorf tube per animal. **Lung:** One 1.5-ml eppendorf tube per animal. For isolated tissues the following observations shall be made and recorded:

vas deferens: Note whether there is an unusual amount of sperm or if it is empty. The criteria is whether the vas is pale white, solidly white or swollen dense white.

Bone marrow: Note before isolating the cells whether the bone marrow is red in the femur or is pale indicating anemia.

Forestomach: Note if there are any obvious pre-neoplastic nodules.

Isolation of Bone Marrow Cells from Femurs from Mice

1. Take the femur as close to the joints as possible and remove muscles and tendons.

2. Clip of the ends of the bone so that the marrow is visible.

3. Using a tuberculin syringe with PBS (300-500 µl), squirt PBS through the bone so that the marrow extrudes into a plastic petri dish.

4. Rinse inside of bone with PBS. For mutation analysis, collect marrow from both leg bones and combine into the same dish.

5. With syringe re-suspend marrow in PBS and transfer to microfuge tube. Pellet cells and discard sup.

6. Freeze on dry ice and store at -70.