RANGE-FINDING PROTOCOL FOR IMMUNOLOGICAL EVALUATION* OF SODIUM DICHROMATE (HEXAVALENT CHROMIUM)

ADMINISTERED IN THE DRINKING WATER TO FEMALE B6C3F1 MICE FOR 28 DAYS

BACKGROUND: Sodium dichromate (hexavalent Cr) is a potent sensitizer and individuals can become allergic to SDD by working with it or by being exposed through constant contact of Cr containing stainless steel. Sodium dichromate has been shown to be immunotoxic in experimental animals. Rats exposed to 25 to 200 μg of $Cr(VI)/m^3$ as sodium dichromate by whole body inhalation exposure for 28 or 90 days had increased spleen weights and increased antibody response to sheep red blood cells. Changes in immunoglobulin concentration, granulocytes, macrophages and lymphocytes have been observed in bronchoalveolar lavage fluid from exposed animals.

PURPOSE: The purpose of these studies is to determine the potential effects of SDD on the immune system when administered in the drinking water and to establish the doses to be used in a full immunotoxicology protocol.

TEST ARTICLE: Sodium dichromate dihydrate (CAS# 7789-12-0; Cat#398063) (Mol. Wt. 297.96)

TEST ARTICLE STABILITY: The test article stability was determined by the National Institute of Environmental Health Sciences. Formulation stability was at least 42 days when sealed and stored at room temperature and protected from light.

TEST ARTICLE PREPARATION: Sodium dichromate dihydrate solutions will be prepared every two weeks and stored refrigerated and protected from light. Water bottles will be changed and filled twice per week.

DOSES TO BE ADMINISTERED:. Five concentrations Sodium dichromate dihydrate (0, 16.125, 31.25, 62.5, 125, and 250 ppm in drinking water) will be utilized. .

ROUTE OF ADMINISTRATION: The Test Article will be administered in the drinking water using Large Amber Screw Top Septum bottles with Teflon/silicone septa (Sigma).

VEHICLE CONTROL VH: Tap water will be used as the vehicle control. Vehicle control animals will receive tap water from the same type of bottle used for the Test Article.

POSITIVE CONTROL PC1: Cyclophosphamide (CPS; Sigma Chemical Co.; CAS #6055-19-2), will be given as a positive control to produce expected changes in selected organ weights, hematological parameters, B-cell, T-cell and T-cell subset surface markers, spleen IgM antibody-forming cell response, and the mixed leukocyte response. The positive control animals will receive 50 mg/kg of CPS on the last 4 days of the treatment period by i.p. injection. CPS will be prepared in phosphate buffered saline and stored at -20°C in Room 2018 in Strauss Building (SOP/TAP/026). Positive control animals will receive 0.1 ml/10 grams of body weight.

POSITIVE CONTROL PC2: Anti Asialo GM1 (Rabbit) antibody (Wako BioProducts; Cat#986-10001; stored at 4-8°C) will be given as a positive control in the natural killer cell assay. The positive control animals will receive 0.2 ml of a 1:10 dilution by intravenous injection 24 hours prior to evaluation of Natural Killer (NK) cell activity. Anti Asialo GM1 will be diluted

in sterile physiological saline.

STUDY DURATION: All animals will be exposed to the test article for 28 days. The animals will be evaluated for the appropriate immunological and toxicological parameters on day 29.

SPECIES SELECTION: Female B6C3F1 mice will be used in this study. The mouse is currently the species of choice in conducting immunological studies. The major components of the immune system in the mouse and humans are the same. Agents that perturb the immune system in humans perturb the immune system in the mouse in a similar manner. The mouse has previously been found to be sensitive to the effects of various environmental chemicals on the immune system.

TEST SYSTEM: Animals will arrive at 4-6 weeks of age. The body weight range of animals placed on study will be between 19-23 grams. Upon arrival the mice will be quarantined for at least 5-7 days prior to commencement of treatment. All mice will be randomized using an Apple computer generated randomization procedure. Mice will be individually identified by tattoo and cage card. Mice will be housed 4 animals per cage in plastic shoe box cages with sawdust (hardwood) bedding and maintained on Harlan Teklad Laboratory Diets (NIH 07), the NIH required diet and tap water *ad libitum* from water bottles. The cages will be covered with filter bonnets. The temperature will be maintained at 64.4-78.8°F and the relative humidity between 40 and 70%. The light/dark cycle will be maintained on 12-hour intervals. Mouse cages will be cleaned and sanitized two times per week.

EXPERIMENTAL DESIGN: Following randomization, animals will be assigned to one of the following treatment groups in either an immunology study or a toxicology study. Each vehicle and SDD treatment group will consist of 8 animals. The positive control groups will consist of 8 animals per group. The treatment groups and their designations are shown in the table below:

Group	Treatment	Concentration (ppm)	Designation
1	Vehicle	0	VH
2	SDD	16.125	D1
3	SDD	31.25	D2
4	SDD	62.5	D3
5	SDD	125	D4
6	SDD	250	D5
7	CPS	50 mg/kg	PC1
8	Anti Asialo GM1	1:10 Dilution	PC2

PARAMETERS TO BE MONITORED:

1. General

   a. Body Weight. Animals will be weighed on the first day of treatment, on day 8, 15, 22 and day 29, the day of sacrifice).

   b. Observations. Animals will be observed at the time of weighing and the time drinking water bottles are changed for any pharmacotoxicological signs.

   c. Unscheduled Deaths: Animals will be necropsied.

2. Standard Toxicology Section

a. Scheduled Sacrifice: On the day of sacrifice, a necropsy will be performed. Terminal body weights and organ weights (spleen, thymus, liver, lungs, and kidneys with adrenals) will be recorded.

b. All animals will be necropsied and any abnormalities in spleen, thymus, liver, lungs, and kidneys with adrenals will be noted. Spleen, bone marrow and lymph node will be fixed and stored for possible histopathology.

c. Hematology (erythrocytes, leukocytes, hematocrit, hemoglobin, MCV, MCH, MCHC, platelets and reticulocytes)).

d. Leukocyte differentials .

3. Immunology Section

a. Spleen IgM antibody response to a T-dependent antigen, sheep erythrocytes (day 4 response).

b. Cell Quantification: T cell and T subsets & B cell enumeration, NK cells and Monocytes .

c. Natural killer cell assay

d. Mixed leukocyte response to DBA/2 spleen cells (day 5)

e. Mouse Serum IgM ELISA using sRBC Antigen. Serum from mice used for the AFC assay will be evaluated for the primary IgM levels to sRBC.

Concomitant Studies: The table below shows the planned parameters that will occur for each study.

	Parameters Measured			
Study No.	1	2	3	4
1	Cell Quantification	Natural Killer Cell	Mixed Lymphocyte Response	Body Weights
2	IgM AFC to sRBC	Serum IgM to sRBC	Body Weights	
3	Organ Weights; Gross Pathology	Hematology	Histopathology	Body Weights

STATISTICAL ANALYSES: The data obtained in this study will be first tested for homogeneity of variances using the Bartlett's Chi Square Test[2]. Homogeneous data will be evaluated by a parametric one-way analysis of variance[3]. When significant differences occur, treatment groups will be compared to vehicle control using the Dunnett's t Test[4]. Non-homogeneous data will be evaluated using a non-parametric analysis of variance. When significant differences occur, treatment groups will be compared to vehicle control (VH) using the Wilcoxon Rank Test when appropriate[5]. The Jonckheere's Test will be used to test for dose related trends across the vehicle (VH) and the SDD treatment groups[6]. The Student's t Test will be used to compare the positive control for the assay with the vehicle control[7].

*See additional information on NTP general immunotoxicity test protocols

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