West Virginia Chemical Spill: Prenatal Developmental Toxicity Study

Materials and Methods

The National Toxicology Program (NTP) conducted studies in which 4-methylcyclohexanemethanol (MCHM), the primary chemical involved in the Elk River spill, was given to pregnant rats to study the effects on prenatal development of their offspring.

Experimental Design

A total of 125 nulliparous, time mated female Sprague Dawley rats (Hsd:SD) were received at Southern Research (SR) from Harlan Laboratories, Inc. (Indianapolis, IN). Animals were approximately 12-13 weeks of age upon receipt. The rats were quarantined upon arrival at Southern Research. Prior to study start, the animals were observed for general health and acceptability for use in this study. All animals were deemed healthy and were included in this study. Based on the results of the health check evaluation, the laboratory veterinarian released the animals from quarantine.

Twenty five time mated females (Sprague-Dawley (Hsd:SD) Animal Source Harlan Laboratories, Inc. (Indianapolis, IN)) per group (Gestation day [GD] 0 = day evidence of mating is observed) were administered doses 0, 50, 100, 200, 400 mg/kg/day via oral gavage. All animals were weighed on arrival, on GD 3, and daily during gestation beginning on GD 6 through GD 21. The dosing sequence of control and dose groups for each dosing day was randomized to avoid a control first and high dose last bias. Animals were dosed between 0700 and 1200. Dose volumes were based on the most recent recorded body weight. Adjusted maternal body weight was calculated by subtracting the weight of the gravid uterus from the GD 21 body weight. Adjusted maternal body weight gain (GD 6-21) was calculated using the adjusted maternal body weight. Food consumption for all animals was collected on GD 3, 6, 9, 12, 15, 18, and 21.

All animals were observed (cageside) at least twice daily (before 1000 and after 1400; at least 6 hours apart) throughout the pre-study and study periods for signs of moribundity and mortality. Detailed observations (cage removal) of each animal were performed at least once during the pre-study period and at least once daily while on study (1 to 3 hours post-dose). Animals were provided Irradiated NIH-07 ½" pellets or wafers (Zeigler Bros., Gardners, PA) and Birmingham municipal water was provided *ad libitum* via an automatic watering system.

Cages were lined with irradiated hardwood-chip bedding (Sani Chips®, P.J. Murphy Forest Products, Corp., Montville, NJ), suspended on stainless steel racks, and covered with disposable filters. Standard cleaning procedures included wiping all Cage size and animal care conformed to the guidelines of the *Guide for the Care and Use of Laboratory Animals,*(1) the U.S. Department of Agriculture through the Animal Welfare Act (Public Law 99–198), the Public Health Service Policy on Humane Care and Use of Laboratory Animals (Public Law 99–158).

Adult females were euthanized on GD 21 by CO2 inhalation. Offspring (≥GD 15) were euthanized by intraperitoneal injection of sodium pentobarbital-containing solution followed by bilateral pneumothorax and/or decapitation.

Blood Collection

On GD 21, dams had blood samples collected for hematology and clinical chemistry evaluation. Blood was collected by cardiac puncture at the time of euthanasia, immediately after cessation of vital signs, into tubes containing K3EDTA (hematology samples; approximately 1.0 mL/sample) or no anticoagulant (clinical chemistry samples; approximately 2.0 mL/sample). The time of euthanasia/blood collection was

recorded. The contents of the tubes containing anticoagulant were mixed by gentle inversion upon collection and maintained at room temperature. Samples obtained for hematology and clinical chemistry were analyzed on the day the samples are collected.

Post-Mortem Examinations

A postmortem examination was performed on GD 21 and included gross examination of abdominal and thoracic viscera of the dam. Uteri with visible implantation sites were weighed (with ovaries attached) and the number of corpora lutea in each ovary was counted. The number, type, and position of implantation sites were determined. Uteri with no visible implantation sites were placed in 10% aqueous solution of ammonium sulfide to detect very early resorptions. Samples of the kidneys and liver were preserved in 10% neutral buffered formalin for possible future histopathology along with animal identification. Residual carcasses and tissues were discarded without further evaluation.

Offspring Examinations

On GD 21, all fetuses were examined for external abnormalities, palatal closure, and sex. The gross appearance of the placentae from all fetuses was recorded. All fetuses were weighed to one hundredths of a gram. All fetuses were then euthanized.

All fetuses were examined for visceral alterations by fresh tissue dissection. For each litter, the first fetus and every other fetus thereafter (approximately half of the fetuses) were selected for soft tissue examination of the head; the heads of these fetuses were removed, placed in Bouin's fixative, and examined by a free-hand sectioning technique. All fetuses were fixed in alcohol, processed, and the skeletons stained with alizarin-red and alcian blue. The skeletal bodies of all the fetuses and the skulls of half the fetuses (fetuses that were not designated for head examination) were examined for bone and cartilage alterations.

All fetal data was collected in a manner that preserved the dam/litter origin and the uterine position of each fetus.