

West Virginia Chemical Spill: 5-Day Toxicogenomic Studies: *In Vivo* Micronucleus Assay Component February 2015 NTP Update

Synopsis

The National Toxicology Program (NTP)¹ evaluated three chemicals that were spilled into the Elk River in West Virginia for their ability to cause genetic damage by using an assay that measures the induction of micronuclei in rat red blood cells (erythrocytes). Micronuclei contain small fragments of genetic material, and their presence in red blood cells is an indication of the capacity of a chemical to cause damage to chromosomes. NTP found that none of the chemicals caused genetic damage by increasing the frequency of red blood cells with detectable micronuclei after oral treatment of male rats for five days.

The chemicals tested included: 4-methylcyclohexanemethanol (MCHM), the primary chemical in the spilled liquid; propylene glycol phenyl ether (PPH); and crude MCHM, a commercial mixture containing primarily MCHM along with lesser amounts of other spilled chemicals. NTP is conducting additional studies to evaluate the ability of the spilled chemicals to cause genetic damage in bacteria, and the results will be reported in a future update.

Background

The *in vivo* rodent erythrocyte micronucleus assay has been used widely for over 30 years as a standard test to evaluate the potential of a chemical or other environmental exposure (e.g., radiation) to cause genetic damage. This assay assesses a chemical's ability to cause chromosomal damage, measured as micronuclei, in rapidly dividing precursors of red blood cells in the bone marrow. Although the genetic damage initially occurs in red blood cells in the bone marrow, the cells move from the bone marrow into the blood as they mature. Thus, either bone marrow or blood samples can be used to measure the frequency of micronucleated red blood cells. Chemicals that induce micronuclei in red blood cells of rodents have a high likelihood of causing cancer in rodents.²

Findings from the *In Vivo* Micronucleus Assay

NTP conducted a 5-day study in rats to evaluate the ability of three of the chemicals spilled into the West Virginia Elk River to cause chromosomal damage. These chemicals were 4-methylcyclohexanemethanol (MCHM), propylene glycol phenyl ether (PPH), and crude MCHM, a commercial mixture containing more than 70 percent MCHM along with lesser concentrations of other spill chemicals. Each chemical was mixed in corn oil and administered orally once daily for five days to separate groups of male rats at the following doses:

¹ NTP is a federal, interagency program whose goal is to safeguard the public by identifying substances in the environment that may affect human health. NTP is headquartered at the National Institute of Environmental Health Sciences, which is part of the National Institutes of Health. For more information about NTP and its programs, visit <http://ntp.niehs.nih.gov/>

² Witt KL, Knapton A, Wehr CM, Hook GJ, Mirsalis J, Shelby MD, MacGregor JT. Micronucleated erythrocyte frequency in peripheral blood of B6C3F(1) mice from short-term, prechronic, and chronic studies of the NTP carcinogenesis bioassay program. *Environ Mol Mutagen.* 2000;36(3):163-94.

Chemical	Doses (mg/kg) ^a
Crude MCHM	0, 0.1, 1, 10, 100, 300, 500
MCHM pure	0, 0.1, 1, 10, 100, 300, 500
PPH	0, 1, 10, 100, 500, 1000

^a Six rats per dose level

Blood samples were obtained from the rats 24 hours after the fifth treatment, and the frequency of micronucleated red blood cells was measured using standard procedures.³ The results for all three chemicals were negative; none of these chemicals increased the frequency of micronucleated red blood cells in male rats after oral administration for five days.

Next Steps

In the *in vivo* rodent erythrocyte micronucleus assay, the assessment of genetic damage following chemical exposure is restricted to cells originating in the bone marrow. Because some chemicals may not get to the bone marrow or may cause genetic damage by other means, NTP usually conducts more than one type of study to assess genetic toxicity. NTP is also evaluating the ability of the chemicals in the Elk River spill to induce mutations (changes in DNA) in the bacteria *Salmonella typhimurium* and *Escherichia coli*, also known as the Ames assay, and those results will be reported in a future update.

³ <http://ntp.niehs.nih.gov/testing/types/genetic/invivo/mn/index.html>