

West Virginia Chemical Spill: Bacterial Mutagenicity Study

June 2015 NTP Update

Synopsis

The National Toxicology Program (NTP)¹ tested eight chemicals spilled into the Elk River in West Virginia for their ability to cause mutations, or permanent changes in DNA sequence, using the bacterial mutagenicity or Ames test. The Ames test assesses the ability of a chemical to induce mutations in any of several different strains of bacteria. A positive test in any strain indicates the chemical is mutagenic and, therefore, has the potential to cause cancer. NTP found that none of the chemicals, including 4-methylcyclohexanemethanol (MCHM), the primary component of the spilled liquid, caused mutations in any of the bacterial strains that were used in the test.

In addition to MCHM, the chemicals tested in the Ames assay were propylene glycol phenyl ether, dipropylene glycol phenyl ether (DiPPH), methyl 4-methylcyclohexanecarboxylate, 4-(methoxymethyl)cyclohexanemethanol, and 2-methylcyclohexanemethanol, all constituents of the spilled liquid; a commercial mixture of DiPPH isomers called Dowanol DiPPH glycol ether; and a commercial mixture “crude MCHM” containing primarily MCHM and lesser amounts of other spilled chemicals.

Bacterial Mutagenicity Study

Background on the Bacterial Mutagenicity Study

Bacterial mutagenicity (Ames) tests have been used widely for many years to determine if a chemical has the potential to cause mutations, which are permanent changes in the DNA sequence of the bacteria. These tests are performed regularly in the chemical and pharmaceutical industries and are accepted by regulatory agencies as a standard method of assessing the mutagenic potential of chemicals.

The Ames test employs several different strains of bacteria. NTP routinely uses three strains of bacteria in the test: two strains of *Salmonella typhimurium* and one strain of *Escherichia coli*. Each strain may react differently to chemical exposure, so using multiple strains increases the opportunity for detecting a mutagenic chemical. Chemicals are tested using five or more widely spaced concentrations that are determined by preliminary trials in each bacterial strain.

Study Findings

NTP tested the chemicals listed in Table 1 in the Ames assay to determine their ability to mutate bacterial DNA. Each chemical was tested at a minimum of five concentrations using standard procedures.² All of the chemicals evaluated in the bacterial mutagenicity studies, including MCHM and crude MCHM, were inactive. None were mutagenic, meaning none caused permanent changes in bacterial DNA sequences. These findings reduce concern related to the carcinogenic potential of the spilled chemicals.

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 Information about the test is available on the NTP Website at <http://ntp.niehs.nih.gov/testing/types/genetic/invitro/sa/index.html>.

Table 1. Elk River Spill Chemicals Tested for Bacterial Mutagenicity

CASRN*	Chemical Name	Doses (ug/plate)	Notes
34885-03-5	4-Methylcyclohexanemethanol (MCHM)	200 - 1000	a
NA	Crude 4-Methylcyclohexanemethanol (Crude MCHM)	200 - 1000	b
770-35-4	Propylene glycol phenyl ether (PPH)	300 - 5000	a
51730-94-0	Dipropylene glycol phenyl ether (DiPPH)	300 - 5000	a
NA	Dowanol DiPPH glycol ether	300 - 5000	c
51181-40-9	Methyl 4-methylcyclohexanecarboxylate	15.6 - 3000	a
98955-27-2	4-(Methoxymethyl)cyclohexanemethanol	500 - 5000	a
2105-40-0	2-Methylcyclohexanemethanol	100 - 2000	a

* CASRN = Chemical Abstracts Service Registry Number. ^aMajor or minor constituent of the spilled liquid (a minor constituent is considered to be approximately 20% or less of the spilled material); ^bA commercial mixture containing >70% MCHM along with lesser amounts of five other chemicals; ^cA proprietary commercial mixture of DiPPH isomers.

Next Steps

The studies to evaluate the mutagenicity of eight of the spilled chemicals in bacteria are complete. Studies on two additional spilled chemicals are being conducted, and their results will be reported separately. NTP will consider the findings reported here in any future, overall assessment of the spilled chemicals.